# Importance of Interactions and Feedbacks for Experimental Microbial Aquatic Communities

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# THESIS SUMMARY

Freshwater ecosystems face increasing anthropogenic pressures despite their importance to provide goods and services to the human population. Therefore, being able to predict consequences of environmental disturbances on the individuals and populations in freshwater communities, and the ecosystem processes they underpin, is a primary challenge for ecologists. These consequences depend on features of the community and on the nature of disturbances (press, pulse or ramping), the type of environmental change (e.g., temperature, nutrient availability, mortality), how multiple environmental changes interact, and also on feedbacks between organisms and their environment.

I characterised five major interconnected challenges to investigate in the context of global change biology research: 1) the stability of ecosystem properties, 2) the presence of multiple environmental disturbances, 3) the possibility to up-scale environmental changes across levels of biological organisation, 4) implications on the predictability of disturbances, 5) and the organism-environment feedback. I addressed these challenges by experimentation with microbial aquatic communities in laboratory conditions. These experiments provided a useful and interesting tool to investigate ecological processes, mechanisms and theories. I conducted two main experiments.

The first experiment consisted of exposing a relatively complex microbial community to four common environmental disturbances in a full-factorial experimental design. Results indicated that increasing the number of disturbances reduced the stability of an ecosystem variable, namely dissolved oxygen concentration. Additionally, one particular disturbance (organic matter addition) had a dominant impact across levels of biological organisations, and interactions among the four disturbances were rare and weak. Nevertheless, predictions of the responses to the environmental disturbances were more accurate when interactions among them were accounted for. This highlights that the same experiment can give different conclusions about the importance of environmental effects depending on if one seeks explanation or to make accurate predictions.

The second experiment consisted of investigating the effects of feedbacks between organisms and their environment. Specifically, I tested a general theory that stronger feedback increases the likelihood of multistability, and increases the strength of nonlinearity and hysteresis in response to environmental change. The strength of feedback was experimentally manipulated by controlling rate of gas exchange inside the experimental ecosystems with gas outside (i.e., in the atmosphere). As predicted, stronger organisms-environment feedback increased nonlinearity and the hysteresis observed with a gradual change of environment, but contrary to predictions there was no increase in likelihood of multistability.

In summary, I provided empirical evidence of the consequences of multiple environmental disturbances and the consequences of organism-environment feedback strength in a microbial aquatic community. These consequences suggest that interactions among environmental disturbances are important for predicting ecosystem dynamics and that further experimental studies, particularly when coupled with system-specific mathematical models, are required to better understand the dynamical consequences of environment-organism feedback strength.

# ZUSAMMENFASSUNG

Süßwasserökosysteme haben weltweit eine wichtige Rolle in der Bereitstellung von Ökosystemdienstleistungen und -produkten für die menschliche Bevölkerung. Trotz dieser Bedeutung sehen sie sich einem zunehmenden anthropogenen Druck ausgesetzt. Eine primäre Herausforderung für Ökologen ist es daher die Folgen von Umweltstörungen für die Individuen und Populationen in Süßwassergemeinschaften und die damit verbundenen Ökosystemprozesse vorhersagen zu können. Diese Folgen hängen von den Merkmalen der Gemeinschaft selber, aber auch der Art der Störung (kurzzeitige vs. langfristige Störung), der Art der Umweltveränderung (z. B. Temperaturanstieg, Eutrophierung, Sterblichkeit), der Wechselwirkung mehrerer Umweltveränderungen sowie der Rückkopplung zwischen Organismen und ihrer Umwelt ab.

Ich habe fünf drängende Fragestellungen bezüglich aquatischer Ökosysteme im Kontext des globalen Wandels untersucht: 1) die Stabilität von Ökosystemeigenschaften, 2) Interaktionen von multiplen Umweltstörungen, 3) das Potential Umweltveränderungen in biologischen Organisationen zu skalieren, 4) die Vorhersagbarkeit des Effekts von Störungen, 5) und die Rückkopplung zwischen Organismen und ihrer Umwelt. Ich habe diese Fragen durch zwei Hauptexperimente mit mikrobiellen aquatischen Gemeinschaften (d.h. Mikrokosmen) unter Laborbedingungen untersucht. Mikrokosmen sind ein nützliches Werkzeug, um ökologische Prozesse, Mechanismen und Theorien empirisch zu prüfen.

Das erste Experiment bestand darin, eine relativ komplexe mikrobielle Gemeinschaft vier allgemeinen Umweltstörungen in einem vollfaktoriellen Versuchsaufbau auszusetzen. Ich konnte zeigen, dass die Erhöhung der Anzahl von Störungen die Stabilität einer Ökosystemvariablen, nämlich der Konzentration von gelöstem Sauerstoff, reduzierte. Zusätzlich hatte eine weitere Störung (organische Substanzzugabe) eine dominierende Wirkung über sämtliche Ebenen der biologischen Organisation hinweg. Die Interaktionen zwischen den vier Störungen waren insgesamt selten und schwach. Dennoch waren die Vorhersagen der Reaktionen auf die Umweltstörungen genauer, wenn Interaktionen

zwischen ihnen berücksichtigt wurden. Dies zeigt, dass das gleiche Experiment unterschiedliche Schlüsse über die Bedeutung von Umwelteinflüssen geben kann, je nachdem, ob man Erklärungen sucht oder Vorhersagen macht.

Das zweite Experiment bestand aus der Untersuchung der Auswirkungen von Rückkopplungen zwischen Organismen und ihrer Umwelt. Insbesondere testete ich die Hypothese, dass eine stärkere Rückkopplung die Wahrscheinlichkeit der Multistabilität und die Stärke von Nichtlinearität und Hysterese als Reaktion auf Umweltveränderungen erhöht. Die Stärke der Rückkopplung wurde experimentell manipuliert, indem die Geschwindigkeit des Gasaustauschs innerhalb der experimentellen Ökosysteme mit der Atmosphäre außerhalb gesteuert wurde. Wie vorhergesagt, erhöhte eine stärkere Rückkopplung zwischen Organismen und ihrer Umwelt die Nichtlinearität und den Hysterese-Effekt bei einer allmählichen Veränderung der Umgebung. Im Gegensatz dazu gab es entgegen der Vorhersage keine Zunahme der Wahrscheinlichkeit der Multistabilität.

Zusammenfassend habe ich empirische Belege für die Folgen von multiplen Umweltstörungen und die Folgen der Feedback-Stärke von Organismen in einer mikrobiellen aquatischen Gemeinschaft erbracht. Diese Folgen legen nahe, dass Interaktionen zwischen Umweltstörungen wichtig für die Vorhersage der Ökosystemdynamik sind und dass weitere experimentelle Studien, insbesondere in Verbindung mit systemspezifischen mathematischen Modellen, erforderlich sind, um die dynamischen Konsequenzen der Rückkopplungsstärke zwischen Organismen und ihrer Umwelt besser zu verstehen.

*Translated from english by Dr. Frank Pennekamp.*

# Chapter 1

# General introduction

## Freshwater ecosystems under global change

Despite occupying less than 1% of the Earth's surface, freshwaters and the biodiversity within them provide invaluable goods and services to people (Figure 1; **provisioning**: drinking water, fisheries; **regulating**: nutrient cycles; **cultural**: fishing; Millenium Ecosystem Assessment *et al.* 2005; Carpenter *et al.* 2009).

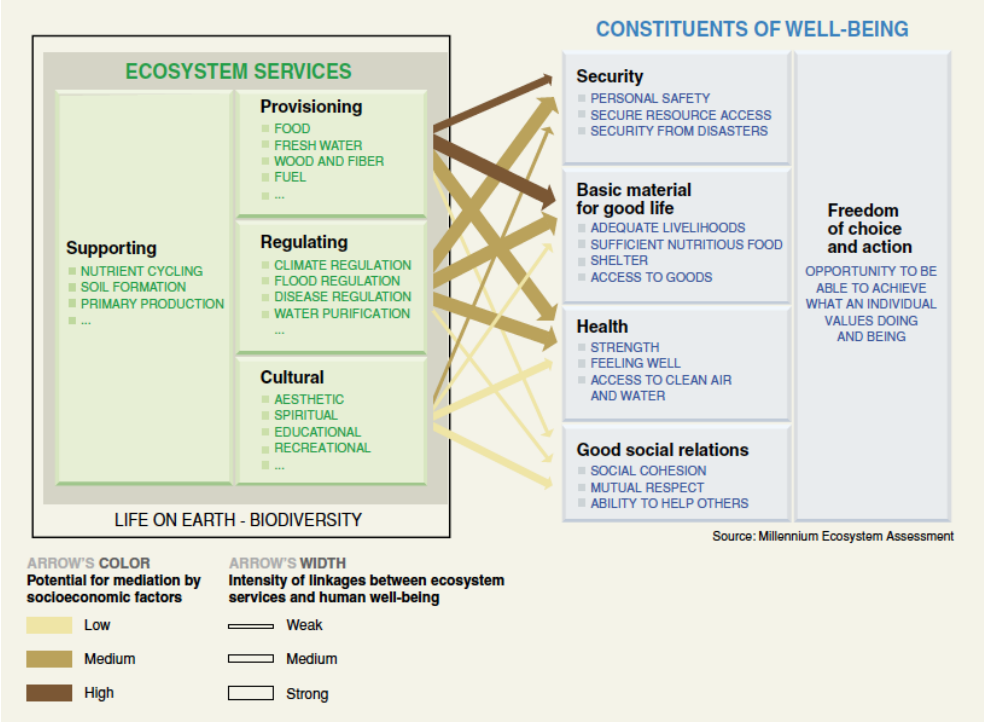


*Figure 1: Linkages among biodiversity, ecosystem services and human well-being. From Millennium Ecosystem Assessment (2005).*

Lakes, rivers, wetlands, and even smaller water bodies such as phytotelmata, are also involved in flows of organisms and materials to and from terrestrial and marine ecosystems (Likens & Bormann 1974; Gounand *et al.* 2017). As such, and alongside other ecosystem types, this makes understanding the structure and functioning of freshwater ecosystems essential (Carpenter *et al.* 1992). Furthermore, research about aquatic ecosystems has sometimes driven conceptual advances in ecological science,

such as the development of theory about alternate stable states, non-linear responses to environmental change, and hysteresis in responses to environmental change that resulted in part from research about shallow lakes (Scheffer & Jeppesen 2007).

Freshwater ecosystems experience disturbances and environmental change, particularly as the demands and requirements of human populations continue to increase. It is possible that some freshwater ecosystems and the organisms within them experience particularly severe disturbances and environmental change, due to features such as proximity to human populations, lack of potential to disperse, and intimate interfaces with terrestrial ecosystems. Hence freshwater ecosystems are among the most stressed ecosystems (Sala *et al.* 2000; Dudgeon *et al.* 2006). They face changes in water chemistry, alteration of their physical environment, the introduction of invasive species, and over-harvesting of the resources, among other environmental changes.

The consequences of such stressors, some of which occur locally, and some of which are regional to global, are already widely observed. For example, the accumulation of several drivers (e.g., overexploitation of resources, land use, pollution, and climate change) led to a loss of more than 20% of the freshwater fish species (Moyle & Leidy 1992)and ∼50% of freshwater vertebrates are declining (Dudgeon *et al.* 2006). These stressors, coupled with loss of diversity, raise the potential to compromise the sustainable delivery of ecosystem services both by direct effects of stressors, and indirect effects mediate via biodiversity loss (Sala *et al.* 2000; Worm *et al.* 2006; Brook *et al.* 2008; Hooper *et al.* 2012). The urgent need to maintain biodiversity and sustainable ecosystem services brings to light several challenges for scientists, managers and policymakers.

# Challenges with global change

### Challenge #1. Stability

Stability largely concerns how populations, communities and ecosystems react to perturbations (Donohue *et al.* 2016). These responses, and the type of perturbation (press, pulse or ramp), combine to give several components of stability. Resistance is by how much an ecosystem property is affected by a perturbation; small effects imply high resistance and high stability. Resilience has two potential meanings: the rate of recovery from the effects of a perturbation (Pimm 1984) and the magnitude of perturbation a system can experience before shifting into a new state or regime (Holling 1973). (These two meanings are used in different places in this thesis, and are then clearly defined.) Temporal variability is a third component of stability, with high temporal variability in an ecosystem property, such as total biomass, indicating low stability. In the absence of any external perturbations, temporal variability can be an indicator of the inherent/intrinsic stability of an ecological system. In a situation with fluctuating environmental conditions (e.g., changing temperatures) then the observed temporal variability of an ecosystem property will result from a combination of features of the system (e.g., interaction strengths), of resistance to the fluctuations, and of resilience to effects of the fluctuations. Overall, when facing a perturbation or fluctuating environmental conditions, an ecosystem is stable if its components are resilient (fast recovery), resistant (small amount of change) and show little temporal variability.

Two major challenges concern the stability of ecosystems functions. The first is how stability varies with biodiversity and includes what if any effect biodiversity has on stability. The relationship between diversity and stability has an enduring and prominent place in ecological research (McCann 2000). Elton (1958) first proposed that less diverse communities would be less stable, after which May (1972) showed that stability could be lowest in more diverse communities. Since then many projects have advanced understanding about the nature of the diversity-stability relationship,

and the mechanism underlying it (e.g., Pimm 1984; McCann 2000; Tilman *et al.* 2006; Ives & Carpenter 2007; Loreau 2010; Loreau & de Mazancourt 2013). Recently, motivated by the predicted loss of biodiversity, the research has shifted to understanding the relationship between diversity and stability of ecosystem function delivery, with a particular focus on the temporal variability (i.e., stability) of ecosystem function, and how resistance and resilience may contribute to this relationship (Loreau 2000; Cottingham *et al.* 2001).

The second challenge is the need to link between theoretical and empirical stability components due to conceptual and practical differences. Theoretical studies often look at ecological systems that return to an equilibrium point (e.g., Neutel *et al.* 2002; Allesina & Tang 2012), which is restrictive as natural systems would face continually and unexpected perturbations, such that they may never return. Furthermore, the asymptotic resilience that many theoretical studies' concern is difficult to measure in practice, and even may be of limited relevance for continually fluctuating systems, where persistence might be a more suitable stability criterion (Arnoldi *et al.* 2016). Hence, in natural systems and laboratory experiments, it is unlikely to observe this asymptotic resilience due to unexpected changes in the environment (e.g., additional perturbation before the full recovery) or time constraint. Hence recent stability research has attempted to bridge these two approaches using stability measurements such as temporal variability that are practically tractable and of considerable relevance for pure and applied ecology science (Arnoldi *et al.* 2016; Donohue *et al.* 2016).

**Challenge #2. Multiple drivers**

It is unlikely that an ecosystem only faces one perturbation at a time (Paine *et al.* 1998). The presence of multiple simultaneous environmental changes is challenging for at least three reasons: 1) the diversity in environmental drivers, 2) the interactions between these drivers, and 3) the diversity of spatiotemporal scales they occur at.

*Diversity in environmental drivers.*

In 1989, global change was described with four horsemen of the ecological apocalypse (habitat loss and fragmentation, over-exploitation, invasive species and co-extinctions) (Diamond *et al.* 1989). Subsequently, climate change was added as a fifth horseman (Dunn *et al.* 2009). Therefore global change drivers have been categorised into five main classes: climate change, land use change, pollution, invasive species, and over-exploitation (Millenium Ecosystem Assessment *et al.* 2005). Each global change driver has the potential to reduce the biodiversity on all biomes (Sala *et al.* 2000), especially on freshwater ecosystems (Dudgeon *et al.* 2006). The challenge within the diversity in drivers is that each driver likely has different impacts within and across levels of ecological organisation (see Table 1).

*Table 1: Effects of global change drivers on freshwater ecosystems at different levels of biological organisation. This is a non-exhaustive review of ecological responses, from the main references. We selected the references according to a literature search containing "freshwater" AND "biological organisation" AND the driver; and selected the most cited publication.*

| | **Individual** | **Population** | **Community** | **Ecosystem** | *References* |
|---|---|---|---|---|---|
| **Climate change** (warming) | metabolism, growth rate, body size, foraging behaviour | extinction | loss of top predator, interaction strength, size spectrum, size-structured food webs, composition | biomass, respiration, decomposition, nutrient cycling, benthic-pelagic coupling, resource assimilation | Woodward *et al.* 2010 |
| **Pollution** | body size, survival, growth rate | population size, extinction | composition and structure | biomass, catastrophic shift, transfer of contaminant to organisms in higher trophic position | Sala *et al.* 2000; Dudgeon *et al.* 2006 |
| **Invasive species** | survival of native species, behaviour (predation or competition), habitat use, foraging | abundance, distribution | composition and structure, direct and indirect inter-specific interaction | path-way and magnitude of movements of energy and nutrients | Simon & Townsend 2003; Dudgeon *et al.* 2006 |
| **Land use** | | dispersal, extinction | | water quality | Sala *et al.* 2000; Foley *et al.* 2005 |
| **Over-exploitation** | body size, sexual maturity | population size, extinction | loss of top predator, composition and structure, inter-specific interaction | | Sala *et al.* 2000; Dudgeon *et al.* 2006; Geist 2011 |

*Interaction between environmental drivers.*

An important challenge is the accumulation of global change drivers that have the potential to interact with each other (e.g., Brook *et al.* 2008). Most of the predictions about cumulative impact of global change drivers used the additive hypothesis (e.g., Sala *et al.* 2000; Jetz *et al.* 2007; Halpern *et al.* 2008). This hypothesis is that the total effect of several drivers will be the sum of their individual effects (Figure 2). This hypothesis has the advantage of being relatively simple and can use the extensive research on individual drivers. Nevertheless, any interactions among drives will limit the value of this hypothesis, and there is emerging, though sparse, information that interactions may be prevalent and significant (Jackson *et al.* 2016). In such cases, the additive hypothesis will over- or under-estimate the real impact of multiple simultaneous environmental drivers.

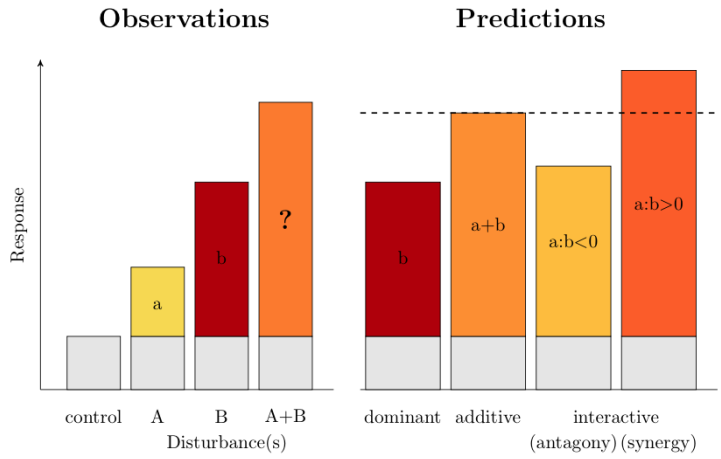


*Figure 2: How to predict the cumulative impact of environmental change drivers? The additive hypothesis suggests that the cumulative impact can be the sum of individual effects (a and b), while non-additive hypotheses include antagonistic or synergistic interaction (a:b <0 or a:b >0 respectively). The dominant hypothesis is a specificity of antagonistic interaction.*

Overestimation of cumulative impact would be observed when antagonistic (negative) interaction occurs (Figure 2). One specific type of antagonistic interaction is when the combined effect of multiple drivers equals the size of the largest individual

effect; this is sometimes termed the dominant hypothesis. Underestimation of cumulative impact is observed when synergistic (positive) interactions occur (Figure 2). The presence of both antagonistic and synergistic interactions can have implications for predicting the effects of environmental change as then can imply ecological surprises (Christensen *et al.* 2006; Jackson *et al.* 2016).

A further challenge is associated with the logistics of researching anything much greater than interactions among two drivers. Such research ideal will involve factorial variation of multiple drivers, which can very quickly lead to very large and impractical experiments or observational studies. Hence, studies of interactions tend to be limited to two or three drivers, while real ecosystems face much more than this.

*Spatio-temporal scales.*

To make things worse, multiple drivers can be observed at different spatial and temporal scales (Levin 1992). While global change is called "global" because the consequences are observed on all biomes, their magnitude differs at smaller scales (local, regional or biome; Figure 3). For example, the global rising temperature is expected to have greater impact at higher latitude (especially on the permafrost biome), while land use change will mostly affect tropical biome (Sala *et al.* 2000).

Press, pulse and ramp perturbations are three types of perturbations that differ in their temporal scale and hence have the potential to differentially affect ecosystem components (Lake 2000). A pulse is a sudden perturbation that can occur frequently or unexpectedly but does not last (e.g., heat wave, temporary flooding). A press perturbation remains over time (e.g., nutrient loading). A ramp perturbation is characterised by gradual increasing or decreasing environmental change (e.g., temperature increase), therefore the perturbation increases in strength over time (Lake 2000). If not removed or attenuated, the ecological system faces long-term perturbation that profoundly affects the entire ecosystem components (Scheffer *et al.* 2001).

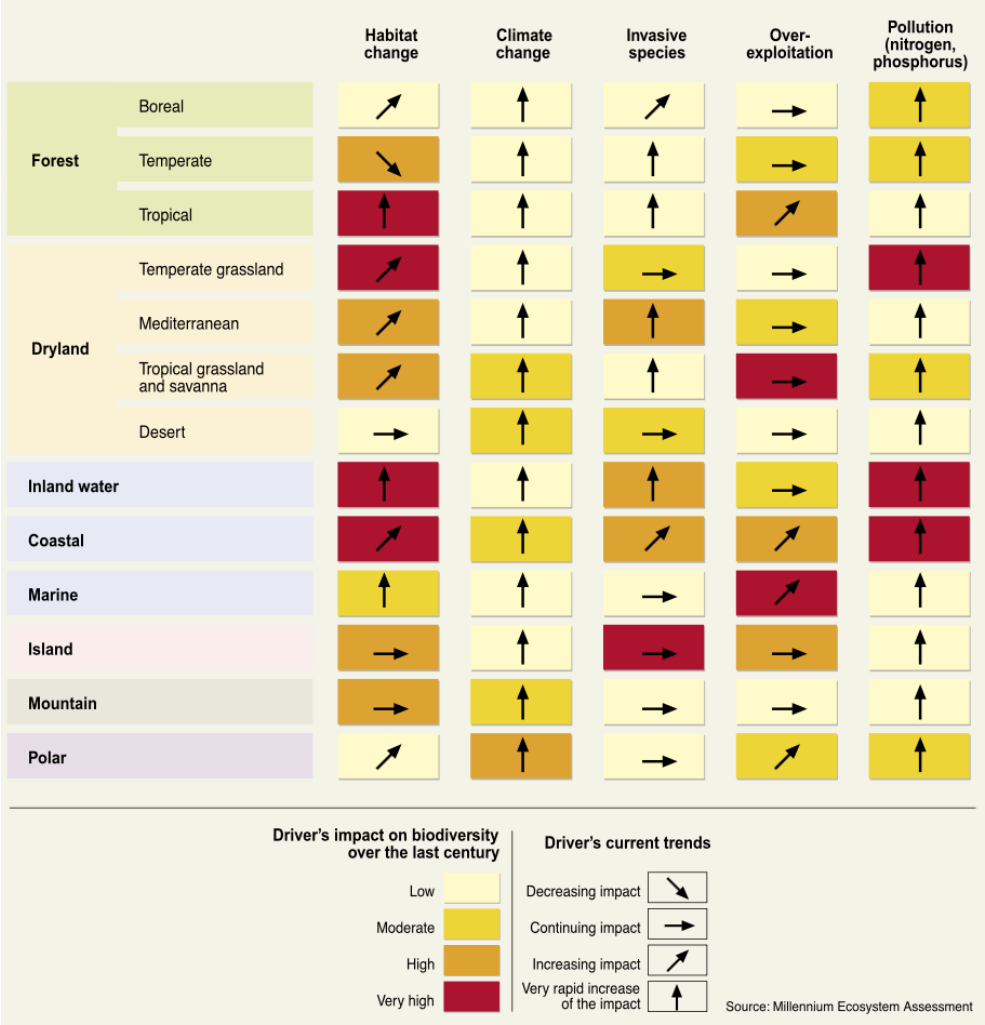


*Figure 3: Drivers impact on biodiversity over the last century and their current trend. From Millennium Ecosystem Assessment (2005).*

### Challenge #3. Regime shifts. non-linearity and feedbacks

With ecosystem facing long-term perturbations, the ecosystem state might shift to another state that is not so desirable in term of the ecosystem services provided or the amount and type of biodiversity (Scheffer *et al.* 2003). For example, with increased nutrient loading, some aquatic ecosystems show regime shift from a clear water and macrophyte state to turbid water state dominated by microbial autotrophs with anoxic

conditions that many organisms cannot tolerate (e.g., Carpenter *et al.* 1999; Scheffer *et al.* 2001). These regime shifts are often abrupt relative to the change in nutrient loading, such that one observes very non-linear responses of ecosystem variables to nutrient loading, and more generally to an environmental gradient. Other features of these regime shifts can be alternative stable states and hysteresis. Alternate stable states are when two communities can be observed for a same environmental condition. And hysteresis is when the alternative stable state depends on historical dependency.

Regime shifts, nonlinearities, hysteresis, and alternate stable states can be caused by feedbacks within the ecosystem, in particular by the presence of positive feedbacks that pull the system towards an attractor/regime/state (synonymous here and throughout) and also act to keep the system in the attractor it currently occupies. These feedbacks have great consequences for the predictability of ecological system as they can reinforce (i.e., positive feedback loop) or dampen (i.e., negative feedback loop) the change affecting the loop.

The importance of the feedback has been strengthened since 1925 by Alfred Lokta in population biology as the "organisms and environment should be considered as a single evolving system in which each influences the other." And numerous theoretical studies reinforced Lotka's statement. Nevertheless, few experiments have explicitly tested the role and importance of feedbacks, for example by manipulating their strength.

**Challenge #4. Predictability**

The need of predictions for managing ecological systems raised this topic to a new research area: the predictive ecology (Mouquet *et al.* 2015; Petchey *et al.* 2015; Houlahan *et al.* 2017).

Predictions in ecology can be either explanatory or anticipatory (Pennekamp *et al.* 2016). The explanatory (or corroborative) approach is hypothesis driven and compares the predictions to the observations. Laboratory experiments are an interesting tool to test hypotheses due to their high control and replication (Altermatt *et al.* 2015).

Therefore, explanatory predictions are used to build and advance theory via hypothesis testing. The anticipatory approach concerns predictions about the future state of a system. For example, the IPCC predicted the potential future temperature increase based on economic scenarios of CO2 emissions (IPCC *et al.* 2007); or predictions about the probability of extinction of a particular population (e.g., Botkin *et al.* 2007).

These two approaches are intrinsically correlated as improving explanatory predictions will improve, in theory, the understanding and knowledge of how systems work, and thus the models used to make anticipatory forecasts.

**Challenge #5. Up-scaling**

The attempt of scaling observations from small to large scales aims to link processes to patterns at different levels of complexity and therefore to build theories and find a unified solution (Levin 1992). Understanding pattern is critical to upscale processes to broad extents for conservation and management of ecological systems (e.g., Lodge *et al.* 1998). In ecology, scaling processes can be of two types. The first is a spatial up-scaling from local to regional or global. For example, to understand how landscape setting was related to the distribution and connectivity patterns in freshwater ecosystems (Fergus *et al.* 2017). The second is an organisational up-scaling, from genes or individuals to ecosystems functioning. For example, the metabolic theory of ecology (Brown *et al.* 2004) could explain patterns in life history attributes, population interactions, and ecosystem processes with the organisms' metabolic rates that depend on body size and temperature. Another approach is to parameterize models to explain higher scales using information from smaller scales. For example, explaining ecosystem functions with community composition (Suding *et al.* 2008) or community dynamics with morphological and behavioral traits (Griffiths *et al.* 2017) that improved the predictability of the models.

# Overview of my Ph.D. research

Answering all of these challenges would require more than one person doing more than one Ph.D.; therefore my Ph.D. research focused on how aquatic microbial community reacts to multiple environmental disturbances, and how the community feedbacks on its environment. Nevertheless, I also touched on the other three challenges, stability, prediction, and scaling, as they often have implications for each other.

The environment shapes community composition according to ecological niche theory (Hutchinson & MacArthur 1959; Denny & Benedetti-Cecchi 2012). Indeed, the environment can be decomposed in a multitude of resource niches, for which species compete (i.e., interspecific competition). While the functional diversity and trophic position will determine the community structure. The ecosystem functions, on what ecosystem services rely, are affected by the community composition and structure. Additionally, the community and its organisms interact with their environment (i.e., ecological feedback) by consuming resources, by modifying physically and/or chemically the environment, and by affecting other species (Figure 4).

This Thesis is composed of three research chapters. The first two concern results of one experiment, and the third the results of a second experiment. The first experiment involved a complex microbial community with three trophic levels (six resources, nine consumers and four predators). I applied four common environmental disturbances (increased temperature, increased phosphorus, increased organic matter and decreased light availability) in a replicated full factorial design.

In the first chapter, I investigated the stability of an integrative ecosystem variable, the dissolved oxygen concentration, under four common environmental disturbances in fully-factorial combination. This is one of the first experiments to investigate up to four-way interactions among environmental changes. I measured the strength of the interactions relative to the main effects, and whether any interactions were antagonistic or synergistic. I also tested whether the interactions between these disturbances affect

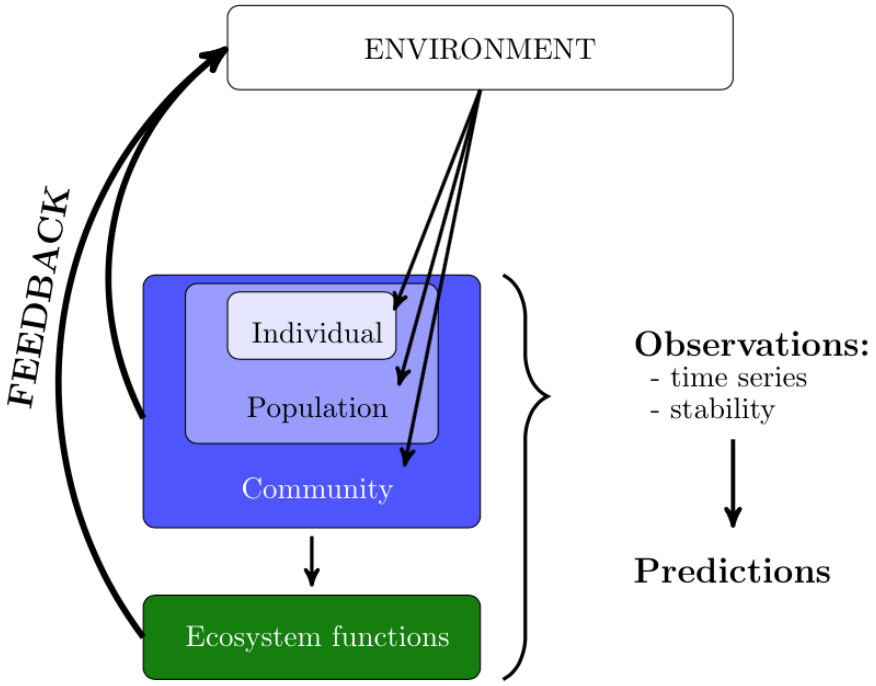


*Figure 4: General context of my Ph.D. research.*

the predictability of three stability components (resistance, resilience and return time). This chapter, "Temporal scale dependent interactions between multiple environmental disturbances in microcosm ecosystems" was published in the journal Global Change Biology in 2017.

In the second chapter, I used results of the same experiment to investigate the effects of multiple environmental disturbances across levels of ecological organisation, including individual traits (morphological and behavioural traits), population dynamics, community composition and structure, and ecosystem functions (decomposition, biomass, respiration and net primary production).

The final research chapter describes a novel study of the importance and influence of feedback strength between the organisms and their environment. I conducted a second experiment that involved a simpler community than the first, though also with

three trophic levels (combining two resources, two consumers and one predator). I manipulated the feedback strength by controlling the gas exchange rate between the atmosphere and the microcosm, and I gradually increasing and decreasing the temperature to test for effects of feedback strength on non-linearity and hysteresis.

## Study system: ciliates community in microcosms

At the base of aquatic food webs, microbial and planktonic communities contribute to the structure of aquatic ecosystems and biogeochemical fluxes (Cotner & Biddanda 2002; Azam & Malfatti 2007). Within the micro-zooplankton (heterotrophic planktonic organisms with a size range of 20-200µm (Edwards & Burkill 1995)), ciliates and heterotrophic dinoflagellates are numerically the most important components (Capriulo *et al.* 1991). With their importance in aquatic ecosystems and their dominance in communities, Ciliates are also used as bioindicators of water quality and ecosystem state (Foissner & Berger 1996; Payne 2013).

Briefly, Ciliates protozoa are free-living unicellular organisms, globally distributed, that show an impressive diversity in terms of morphological traits (more than 3000 morpho-species; Foissner 1999), behaviours, feeding strategies (bacterivorous, algivorous, omnivorous, predator and decomposers), and reproduction types (clonal and/or sexual) (Fenchel 1987).

Given their diversity and their short generation time, using microbial communities containing ciliates protozoa allows a wide range of experimental designs and methods to test ecological and evolutionary theories with replicated and controlled systems, which are needed to validate model assumption and to test model predictions (Altermatt *et al.* 2015). For example, Gause tested the concepts of exclusion competition and predation - two major concepts shaping the community structure and composition - using three protists species (*Paramecium caudatum* and *P. aurelia*; *P. caudatum* and *Didinium*

*nasutum* respectively; Gause 1934). While the communities in microcosm experiments are often considered simple and relatively homogeneous, they nevertheless can motivate and provide great advances in understanding the structure and functioning of ecological systems.

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# Chapter 2

# Temporal scale dependent interactions between multiple environmental disturbances in microcosm ecosystems.

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**Abstract**

Global environmental change has negative impacts on ecological systems, impacting the stable provision of functions, goods and services. Whereas effects of individual environmental changes (e.g. temperature change or change in resource availability) are reasonably well understood, we lack information about if and how multiple changes interact. We examined interactions among four types of environmental disturbance (temperature, nutrient ratio, carbon enrichment, and light) in a fully factorial design using a microbial aquatic ecosystem and observed responses of dissolved oxygen saturation at three temporal scales (resistance, resilience and return time). We tested whether multiple disturbances combine in a dominant, additive or interactive fashion, and compared the predictability of dissolved oxygen across scales.

Carbon enrichment and shading reduced oxygen concentration in the short-term (i.e. resistance); although no other effect or interaction was statistically significant, resistance decreased as the number of disturbances increased. In the medium-term, only enrichment accelerated recovery, but none of the other effects (including interactions) were significant. In the long-term, enrichment and shading lengthened return times, and we found significant two-way synergistic interactions between disturbances.

The best performing model (dominant, additive or interactive) depended on the temporal scale of response. In the short-term (i.e. for resistance), the dominance model predicted resistance of dissolved oxygen best, due to a large effect of carbon enrichment, whereas none of the models could predict the medium-term (i.e. resilience). The long-term response was best predicted by models including interactions among disturbances. Our results indicate the importance of accounting for the temporal scale of responses when researching the effects of environmental disturbances on ecosystems.

# Introduction

Global environmental change is known to affect ecological systems in harmful ways and threatens the stable provisioning of functions, goods and services that ecosystems provide (Chapin III *et al.* 2000; Daily *et al.* 2000). Among the most important types of global environmental change are habitat loss and fragmentation, over-exploitation, invasive species and co-extinctions, aptly depicted as 'the four horsemen of the ecological apocalypse' (Diamond *et al.* 1989). Added to this evil "quartet" nowadays is climate change, and to make things worse, there exists the potential for synergies between co-occurring environmental changes (Brook *et al.* 2008). Synergies would exacerbate pressure on natural ecosystems and, if they are difficult to predict, could lead to "ecological surprises", with potentially severe and irreversible consequences (Carpenter *et al.* 1992; Heugens *et al.* 2001; Brook *et al.* 2008; Griffen & Drake 2008; Holmstrup *et al.* 2010; Mantyka-Pringle *et al.* 2012) . On the other hand, antagonistic interactions mitigate each other's effect (Folt *et al.* 1999; Brook *et al.* 2008; Crain *et al.* 2008) . A special case of such antagonistic interactions is when the combined effect of multiple environmental disturbances is equal to the largest effect of any of the disturbances when they occur in isolation (Sala *et al.* 2000; Brennan & Collins 2015).

The presence and strength of interactions among multiple environmental disturbances can have large effects on predictions. For example, Sala and collaborators (2000) compared the future global distribution of biodiversity for scenarios with different assumptions about how multiple environmental disturbances combine. The biome in which biodiversity was most threatened depended greatly on whether one assumed additive/synergistic or dominant combining of the effects of multiple environmental disturbances. The study concluded that the most plausible scenario for the future would be between the additive and synergistic hypothesis, and highlights the importance and priority of research about how multiple environmental disturbances combine.

Although numerous conceptual frameworks for discriminating between synergistic

and antagonistic effects exist (Piggott *et al.* 2015), experimental approaches that manipulate environmental disturbances in a factorial manner, which allows to rigorously test for interactive effects, are still rare (but see Doyle *et al.* (2005); Christensen *et al.* (2006); Brown *et al.* (2012); Griffiths *et al.* (2015)). Often these studies concern only interactions between two environmental factors and evidence regarding the occurrence and types of interactions is mixed (Darling & Côté 2008; Jackson *et al.* 2016). To evaluate the reliability of the scenarios for management decisions, we urgently need to understand how important interactions are and how well we can forecast with models that neglect interactions (Côté *et al.* 2016).

Aquatic systems are particularly vulnerable to environmental changes due to their importance for and proximity to human settlements (Jenny *et al.* 2016). Land use changes, invasive species, climate change, nitrogen deposition and atmospheric carbon dioxide are considered major threats for aquatic organisms (Carpenter *et al.* 1992; Sala *et al.* 2000; Stendera *et al.* 2012). Although many of these environmental disturbances were studied individually to understand their consequences at different levels of ecological organisation, studies investigating their effects in combination are rare (Jackson *et al.* 2016).

A key indicator of the health of aquatic ecosystems is dissolved oxygen (DO) (Walker 1979; Wetzel & Uchman 2001; Hanson *et al.* 2006). Dissolved oxygen is a measure of ecosystem productivity that integrates production and respiration across trophic levels and thus estimates a whole-ecosystem response. Change in dissolved oxygen is hence a functional metric that provides the net effect of different processes. Biologically driven processes provide an integrative measure of the ecosystem functioning (Webster & Benfield 1986) over time and across organisms at different organizational levels. Because functional metrics, are independent on the identities of the species in a community, they provide a more generalizable picture than the specific structure of a given community (Denny & Benedetti-Cecchi 2012). Nevertheless, function influences

structure and vice-versa, and both should be considered to assess the integrity of an ecosystem as a whole. Community structure and ecosystem functioning are strongly affected by low dissolved oxygen concentration (i.e. hypoxia), which may be insufficient to support heterotrophic organisms (> 30% saturation needed ; Wu (2002)). Hypoxic environments have become more common in the last three decades (Diaz & Rosenberg 2008; Diaz & Breitburg 2009) due to increased human pressure on freshwater ecosystems (Jenny *et al.* 2016). Temperature, among other factors, affects dissolved oxygen directly and indirectly by affecting its solubility (Garcia & Gordon 1992) as well as the physiology of organisms (Brown *et al.* 2004; Savage *et al.* 2004). In parallel, nutrient input can trigger bacterial growth (eutrophication), potentially leading to hypoxic condition due to excessive bacterial respiration. Moreover, the interaction of increased temperature and nutrients inputs can intensify hypoxic conditions and ultimately lead to fish extinctions (Moran *et al.* 2010). Hence, understanding how dissolved oxygen levels respond to (e.g. their resistance to) environmental disturbances, and their recovery (e.g., resilience and return time) from environmental disturbances is important for understanding and predicting responses of species and community composition.

Maintaining stability of ecosystems is often desired, as only stable ecosystems can provide functions and services (Isbell *et al.* 2015). The ability of ecosystems to buffer disturbances such as induced by global environmental change is therefore an important aspect of ecosystem functioning. Stability may be also a function of time, therefore the temporal scale of the disturbance and the response should be considered (Christensen *et al.* 2006; Donohue *et al.* 2016). We chose to apply disturbances in a press manner (rather than pulse) in which disturbances were instantaneously applied and then maintained. We considered three temporal scales of response: the short-term effect of disturbance on dissolved oxygen (i.e. resistance); in the-medium term the rate of return of dissolved oxygen to control treatment levels (i.e. engineering resilience); and long-term recovery to control treatment levels ("return time") (Pimm 1984). We use the

term "scale" to describe this variation in the temporal extent over which the responses occur, and thus also the temporal scale of the processes underlying the responses.

We studied the effect of four environmental disturbances, and the direction of interactions among them, on dissolved oxygen availability. As factorial manipulations of environmental disturbances are difficult to achieve in the field, we used an aquatic experimental system consisting of a community of algae, bacteria, ciliates and rotifers (Petchey *et al.* 1999; Altermatt *et al.* 2015). We selected temperature, nutrients, carbon enrichment and light availability as experimental environmental disturbances due to their relevance for natural aquatic systems (Carpenter *et al.* 1992; Piehler *et al.* 2004; Llames *et al.* 2009; Stanley *et al.* 2012; Yankova *et al.* 2016), and manipulated these in a factorial design to detect the effect of potential interactions on DO.

Dissolved oxygen concentration is determined by the action of two biological processes, namely the respiration of all organisms, and the photosynthesis of autotrophs. Effects of the four environmental disturbances on DO will therefore be indirect via effects on community respiration and photosynthesis, and one might expect different effects of each disturbance on each process. For example, carbon enrichment should increase growth, biomass, and therefore respiration at least in the short term, with little effect on photosynthesis, leading to decreased DO. Temperature has stronger effects on respiration than photosynthesis (Yvon-Durocher *et al.* 2010) which predicts that increased temperature decreases DO. Shading should decrease DO due to reduced photosynthesis, at least in the short term. In the absence of a quantitative model of the effects of these various disturbances on photosynthesis, respiration, and DO, predictions about how they will interact are difficult to make. Hence, we tested whether multiple environmental disturbances combine according to hypotheses of additivity (combined effect equal to sum of individual effects), synergy (combined effect greater than sum of individual effects), or antagonism (combined effect less than sum of individual effects). A specific form of antagonistic interaction, that of dominance, was also

tested (combined effect equal to the largest individual effect).

Dominant and additive combining of multiple types of disturbance represents a more predictable situation, because then only information from each individual disturbance is required for prediction. In contrast, interactions between disturbances require additional, and potentially difficult to obtain, information about the sign and strength of the interactions. For a particularly model of combining disturbances (e.g. dominant, additive, interactive) we can also ask how predictability changes with the temporal scale of response.

We expected greater predictability at shorter time scales of response, and lower predictability at longer time scales of response due to greater opportunity for indirect effects at longer time scales. Put another way, direct effects should dominate in the short-term, and direct effects should be more additive/dominant in their combinations, with subsequently greater predictability. In the longer-term, indirect effects, such as those mediated via changes in environmental conditions and community composition, create greater opportunities for interactive combinations of effects of environmental disturbances. Such contributions of indirect effects to unpredictability can cause indeterminacy (i.e. unpredictability) of theoretical perturbation experiments and ecological surprises (Doak *et al.* 2008).

# Material and Methods

## Experimental system

Experimental microcosms were sterile 250 mL glass jars containing 100 mL of Protozoan Pellet Medium (PPM) (Lawler & Morin 1993; Altermatt *et al.* 2015). Media consisted of 0.28 g of crushed Protozoan Pellets (Carolina Biological Supply Co., Burlington, N.C., USA) in one litre of Chalkley's medium. Protozoan pellets provide an organic food source (nutrient and carbon) for bacteria and protists (Kaunzinger *et al.* 1998).

Two additional wheat seeds provided a slow-release nutrient-source. Microcosms were placed randomly in six temperature and light controlled incubators with a 16-8 hours light-dark cycle, at an intensity of 5000 lux during light phase.

## Microbial aquatic community

Our aim was to assemble a moderately complex microbial community with multiple species in multiple trophic groups, so a range of ecological processes were occurring. This was accomplished by assembling a community initially composed of two species of bacteria (*Serratia fonticola*, *Bacillus subtilis* – generally used in laboratory experiments with ciliates cultures (Altermatt *et al.* 2015)) though bacterial composition was not subsequently controlled and was likely higher as the sampling was conducted in non-sterile environment, four species of algae (*Chlamydomonas reinhardtii*, *Scenedesmus quadricaula*, *Staurastrum gracile* and *Desmidium swartzii*), one species of rotifer (*Rotifer* sp.), and 12 species of ciliates; one was algivorous (*Nassula aurea*), five were bacterivorous (*Tetrahymena thermophila*, *Colpidium striatum*, *Paramecium caudatum*, *Blepharisma japonicum*, *Euplotes* sp.) and six were omnivorous (*Euplotes daidaleos*, *Frontonia* sp., *Paramecium bursaria*, *Stentor coeruleus*, *Dileptus anser* and *Actinophrys sol*; the last two have a preference for ciliates, flagellates, amoebae and rotifers). Based on results of previous experiments, extinctions of some species will have happened, particularly at the higher trophic levels, leading to a community with more species at lower trophic levels and fewer at high trophic levels (we did not have access to species composition data when this article was prepared).

Before the experiment, all species were cultured in monoculture in 0.28 $g.L^{-1}$ PPM at 20°C. At day 0, all species were combined with different volumes according their trophic position (10 ml for each algae and bacteria species, 2 ml for each ciliate and rotifer species), and topped up with 13.8 ml of 0.28 $g.L^{-1}$ PPM and 100 µL of each nutrient solution ($NH_4Cl$ and $KH_2PO_4$ in $mg.L^{-1}$) to a total of 100 ml per microcosm.

To assure the presence of predators in the system, five individuals of *Stentor coeruleus*, *Dileptus anser* and *Actinophrys sol* were added to all microcosms the day before the perturbation treatments. Samples were taken from which we aimed to estimate the abundance of each species; analyses concerning this data will appear in a subsequent publication (though we do here report some preliminary bacterial abundance data in Figure 5).

## Experimental design

The experiment was four-way fully factorial with two levels of each treatment, with six replicates of each of the 16 treatment combinations making for a total of 96 experimental ecosystems. This constitutes a quite large and time consuming experiment, and with available resources we could not include more than two levels in each treatment.

An important aspect of this design was the choice of the two levels of each treatment, and we provide justification of these choices below. Nevertheless, it is important to note considerable variability in the predicted real change in these environmental disturbances; this variability results from uncertainty about what is likely, but also from variability through spaces (e.g., some locations likely to be warmer than others). With such variability, choosing most realistic treatment levels for any single environmental disturbance is somewhat arbitrary.

Perhaps more important than individual treatment levels are their relative levels. If we unwittingly made one of the four treatments large in magnitude and the other three small, we could accidentally favour the dominance hypothesis, for example. To avoid this problem, we tested several levels of each environmental change in preliminary experiments. Temperature was held constant at 20°C or increased to 25°C during manipulation using temperature controlled incubators. These temperatures were chosen to align with inter-annual variation in summer water temperature in ponds and lakes (Moore *et al.* 1996; Jankowski *et al.* 2006; Yankova *et al.* 2016). Moreover, this

increased temperature (+5°C) falls within the projection of increases in surface water (A1FI scenario, IPCC, 2007). These temperatures may or may not translate into large effects on physiological rates, depending on the temperature response curves of the diverse species in our communities. Lack of knowledge of many of these response curves limited our ability to use such information when deciding treatment levels.

If light availability is not directly a driver of global change, increased dissolved organic matter due to runoffs can result in an increase of turbidity and therefore a decrease of light availability (Anneville *et al.* 2002). Light availability has been shown to affect phytoplankton photosynthesis (Kirk 1983), turbidity (Llames *et al.* 2009) and phytoplankton biodiversity (Stomp *et al.* 2004). A reduction of 25% light availability was previously found to decrease plankton abundance and to increase respiration rate (Llames *et al.* 2009). Therefore, we chose to reduce the light availability by 30% using shade cloth around the microcosms to assure a relative magnitude of the light disturbance in comparison to the other disturbances.

Human activities (e.g., agriculture) have resulted in increased loading of nutrients in freshwater systems that affect community structure and function (Smith *et al.* 1999; Piehler *et al.* 2004). If nitrogen and phosphorus are limiting resources for primary production, high nutrient inputs can lead to eutrophication of natural systems (Carpenter *et al.* 1998). Oligotrophic lakes are characterised by high nitrogen:phosphorus (N:P) ratio, whereas eutrophic lakes have a lower N:P ratio, generally below 20:1 (Wetzel 1983; Downing & Mccauley 1992; Stets & Cotner 2008; Kratina *et al.* 2012). And even within a lake, the N:P ratio can vary seasonally from 8 to 60 (Kolzau *et al.* 2014). We prepared nitrogen and phosphorus solutions to a ratio of 40:1 using $NH_4Cl$ (at 0.460 mg.$L^{-1}$, corresponding to 1.7576 mol.$L^{-1}$ of N) and $KH_2PO_4$ (at 0.010 mg.$L^{-1}$, corresponding to 0.0439 mol.$L^{-1}$ of P) respectively. We manipulated N:P ratios by increasing the amount of phosphorus (0.027 mg.$L^{-1}$ $KH_2PO_4$, corresponding to 0.1185 mol.$L^{-1}$ of P) with the same amount of nitrogen, resulting in N:P = 15:1.

Similar to inorganic nutrients (nitrogen and phosphorus), dissolved organic carbon has increased in aquatic ecosystems due to anthropogenic pressure (Stanley *et al.* 2012; Williams *et al.* 2016). Carbon enrichment consisted of 0.56 $g.L^{-1}$ of PPM, and the low concentration was 0.28 $g.L^{-1}$ of PPM (Lawler & Morin 1993). Importantly, this approximately factor two difference between levels of the carbon enrichment treatment is small relative to many experimental manipulations, which often cover orders of magnitude (Kaunzinger *et al.* 1998), and is small compared to the differences that can occur as a result of inputs into naturally occurring water bodies ($\sim$0.1 $PgC.yr^{-1}$; Regnier *et al.* (2013)). Every three days, we removed 5ml of medium from each experimental unit, and replaced with 5ml of specific medium for the treatment.

During the first week all communities experienced control treatment levels. On the eighth day, we applied a full factorial combination of four press disturbances (temperature, nutrient, carbon enrichment and light). Responses to the perturbations were monitored until dissolved oxygen had returned to control levels in a large majority (90%) of the replicates (this was achieved by 16 days).

## Quantification of dissolved oxygen content

The dissolved oxygen (DO) saturation was measured daily at the end of a light period of 16 hours using a non-invasive method called chemical-optical sensor (Fibox 4 trace, PreSens, Germany; Altermatt *et al.* (2015)). We assessed the net effect of respiration and photosynthesis on DO. Note that 100% DO saturation corresponds to an oxygen partial pressure of 21%.

## Responses variables

We quantified the treatment effects on DO at three time scales: a short-term response (resistance); a medium-term response (resilience); and a long-term response (return time) (Figure 1).

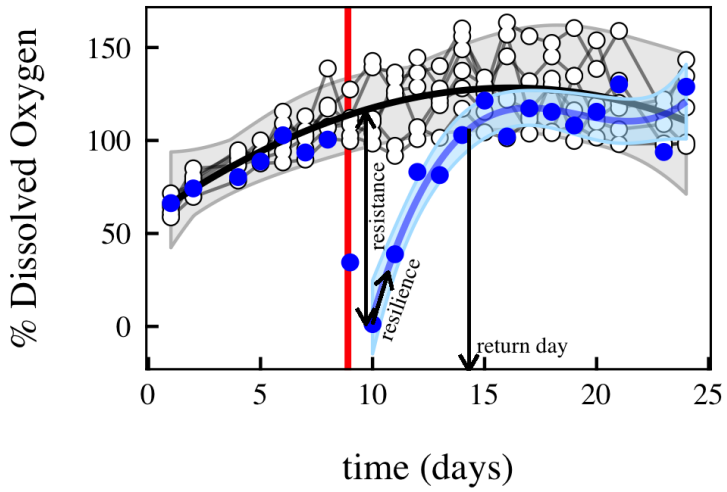


*Figure 1: Illustration of how ecological stability variables i.e. resistance, resilience and return time were measured. The red vertical line shows the time of the disturbance(s). The blue line and points show the dissolved oxygen levels in one treatment replicate (here carbon enrichment). The black line and white points show the six control replicates. The shaded regions show the 95% confidence interval of the control (grey) and treatment (blue) time series.*

Resistance was the effect observed within three days after the perturbation (Pimm 1984). To measure resistance, we determined the maximum difference between DO in a replicate treatment and average DO across the control replicates. We chose a period of three days because visual inspection of the DO time series showed this was long enough to always include the minimum DO caused by the environmental change treatment.

Resilience, in the present study, is considered as the rate of recovery following a perturbation (Pimm 1984), also known as "engineering resilience" (Holling 1996). Theoretically, the resilience is measured as the asymptotic rate of return (Arnoldi *et al.* 2016). Empirical measures of resilience are challenging and less well defined. We estimated the resilience as the rate of change in log difference between a treatment replicate and the average of the control replicates from the day at which DO reached the maximum displacement; this excluded the possibility for system reactivity (Neubert *et al.* 2009) to interfere with our measure of resilience. Calculating the log difference is equivalent to calculating the rate of relative return, rather than absolute rate, rendering the

resilience at least conceptually independent of resistance (Figure 1). The rate of change was estimated by fitting a polynomial of degree three (cubic regression) since this was well supported by the data. Resilience was the first derivative of this polynomial after the system started to return towards DO levels in controlled microcosm (Figure 1).

The return time was estimated as the amount of time taken for DO in a perturbed treatment to recover to the level in control treatments. In practice, this requires accounting for variability in DO among and within control replicates, accomplished by calculating a 95% confidence interval for control DO levels. We also needed to account for variability of DO levels among treatment replicates, again accomplished by calculating a 95% confidence interval around the order-3 polynomial fitted to the return dynamics (the same as used to calculate resilience). Mean return time was the time it took for the mean DO of treatments to fall within the 95% CI of the control, and lower and upper bounds on the return time were when the upper and lower bounds of DO from the treatment 95% CI first fell within the 95% CI of control (Figure 1). If the DO of treatments did not return to within control levels during the experiment, return time was right censored (i.e. the event was not observed at the end of the experiment). In the theoretical setting of exponential return, resilience (rate of exponential return) is the inverse of time to return (Pimm 1984). We did not observe such return dynamics, and analysed resilience and return time independently as they were not correlated (Pearson's r=0.057; t=0.5521, df=93, $P$=0.5822). Furthermore, it is important to note that responses were always relative to average control levels to account for any directional changes in control treatments.

## Statistical analyses

In a first step, resistance, resilience and return time were analysed separately to test for the presence and direction of interactions between environmental change treatments. Explanatory variables were the four treatments: temperature (T), nutrients (N), light

(L), and carbon enrichment (C), each with two levels (control and perturbed) as well as all high-level interactions (Table 1).

*Table 1: Models fit to the observed data, with the data set used to parameterize the models and the numbers of parameters estimated. Notice that the full dataset corresponded to 95 microcosms (instead of 96) because one microcosm (TCL treatment) was removed due to an error in the treatment application.*

| **Hypotheses / models** | **Formula** | **Number of microcosms used to parameterise the models** | **Number of model parameters** |
|---|---|---|---|
| Dominant | ~ Largest main effect | Subset (30) | 5 |
| Additive | ~ T + N + C + L | Subset (30) | 5 |
| Interactive (2-way) | ~ T + N + C + L + all 2-way interactions | Subset (66) | 11 |
| Interactive (3-way) | ~ T + N + C + L + all 2 and 3-way interactions | Subset (89) | 15 |
| Interactive (4-way) | ~ T + N + C + L + all 2, 3 and 4-way interactions | Subset (95) | 16 |

Resistance and resilience were analysed with a linear model using a normal error distribution with the package `stats` (R Core Team 2016), and return times were modelled using survival analysis with the package `survival` (Therneau 2015). The shape parameter of the survival analysis was analysed while the scale parameter was fixed at 1 to avoid lack of convergence. All models were examined visually for the homogeneity of variances and normality and found to follow model assumptions. The significance of effects was tested using two-tailed Type III F- or -test on the global model using maximum likelihood with the package `car` (Fox & Weisberg 2011).

Then we tested the effect of the number of perturbations on each response variable using a mixed linear model with the number of perturbations as an explanatory variable, and the treatments and the replicates as random effects with the package `lme4` (Bates *et al.* 2015). To correct for the fact that a particular treatment could

be involved in different combinations, the overlap between treatments was calculated according to Brennan & Collins (2015) and used as a covariate. The shape of the relationship between the ecosystem response and the number of perturbations can inform the interplay among environmental changes. Additive effects would lead to a linear relationship between the number of perturbations and the ecosystem response while interactive (synergic or antagonist) effects would lead to a non-linear relationship. Finally, the ecosystem response should follow a bimodal distribution when an environmental change would dominate (i.e. with and without the dominant disturbance). Therefore, we first tested the significance of the quadratic term of a polynomial regression to evaluate whether the relationship between the response and the number of perturbations was linear or quadratic. The bimodality of the distribution was investigated using the model including the number of perturbations in interaction with a categorical variable describing the presence of the dominant disturbance in the treatments. The significance of each effect was tested using a two-tailed Type I F-test with the package `lmerTest` (Kuznetsova *et al.* 2016) using the Satterthwaite approximations for denominator degrees of freedom. Examination of full (linear and mixed) models and backward procedures (first removing the interactions) gave the same results.

In a second step, we examined the predictive power of three groups of hypotheses: dominant, additive and interactive (Table 1) using a 2-fold cross validation method. This involved fitting multiple linear models to the first half of the experimental data (test dataset) and then measuring how well the models predicted the second half (validation dataset). We used the adjusted $R^2$ as measure of predictive power. For resistance and resilience, the predictions were the means estimated by the linear models, whereas for return time, the predictions were the time corresponding to 50% of the survival curve. Models varied in the combinations of explanatory variables included, corresponding to (a) a non-additive effect of treatment with only the largest main effect (dominance), (b) one model of additive effects of treatments, and (c) three models of

interactive effects of treatments with up to two, three or four way-interactions (the full model). We only used the minimum data required to parameterize each model: e.g. the additive model used only the experimental data for the main effects, without any interaction treatment combinations (Table 1). To examine the importance of the carbon enrichment treatment, since it appeared to be strong relative to the other treatments, we repeated the entire analysis procedure for the subset of the data that corresponds to performing the three-way factorial experiment with only the temperature, nutrient and light treatments.

Testing models on data to which they were fitted was likely to yield over-optimistic predictive power (over-fitting), therefore we fitted the models to data from three replicates of each treatment combination, and compared their predictions with the other three (or two for TCL). Notice that the full dataset corresponded to 95 microcosms (instead of 96) because one microcosm (TCL treatment) was removed due to an erroneous treatment application. To obtain confidence intervals (95%) of predictive power, we repeated the entire process 1000 times, with replicates randomly assigned to training and test datasets. All statistical analyses were performed in R (R Core Team, 2016).

# Results

At the start of the experiment, DO dynamics were similar across all replicates, including the control, increasing from about 60 to 100% (Figure 2, $F_{15,79}$=0.7439, $P = 0.733$), and then little directional change in control replicates from when the treatments were applied. The DO sometimes exceeded 100% saturation, most likely due to production of oxygen by algal photosynthesis at a faster rate than loss by respiration. As expected, carbon enrichment and shading decreased DO, while increased temperature and changed nutrient ratios had no apparent effect (Figure 3a).

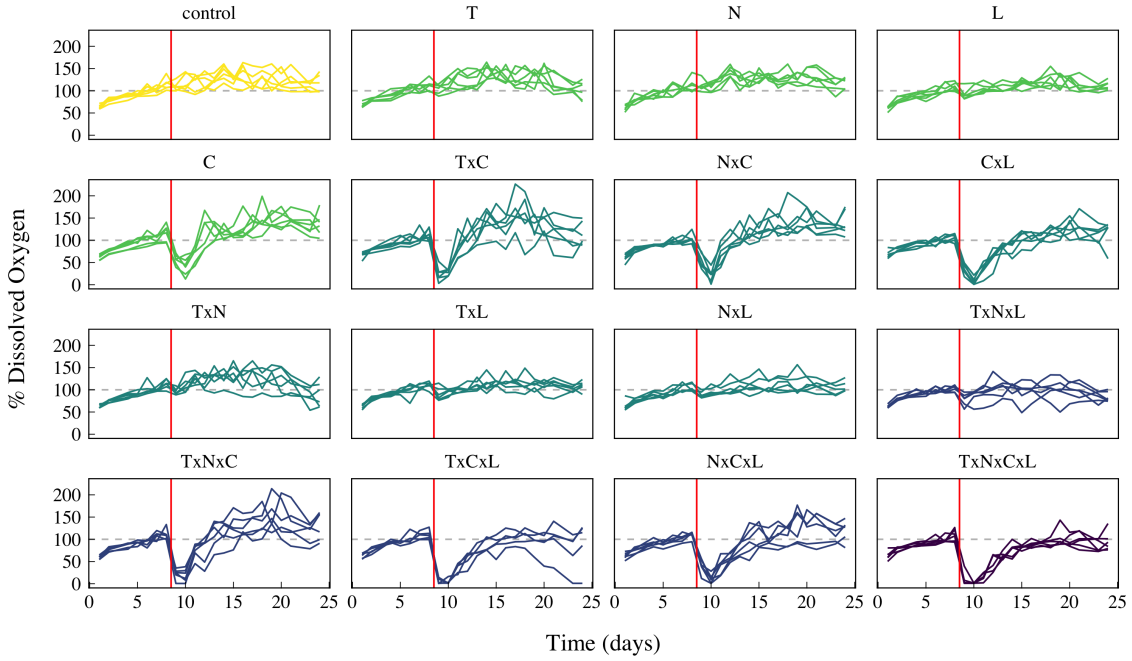


*Figure 2: Time series of dissolved oxygen concentration for all 16 treatments and replicates within each treatment. The red line shows the time of the disturbances (temperature (T), nutrients (N), light (L), and carbon enrichment (C)). The grey dashed line represents 100% oxygen saturation. Colours indicate the number of combined perturbations (yellow = control, green = 1 perturbation, turquoise = 2, blue = 3, purple = 4).*

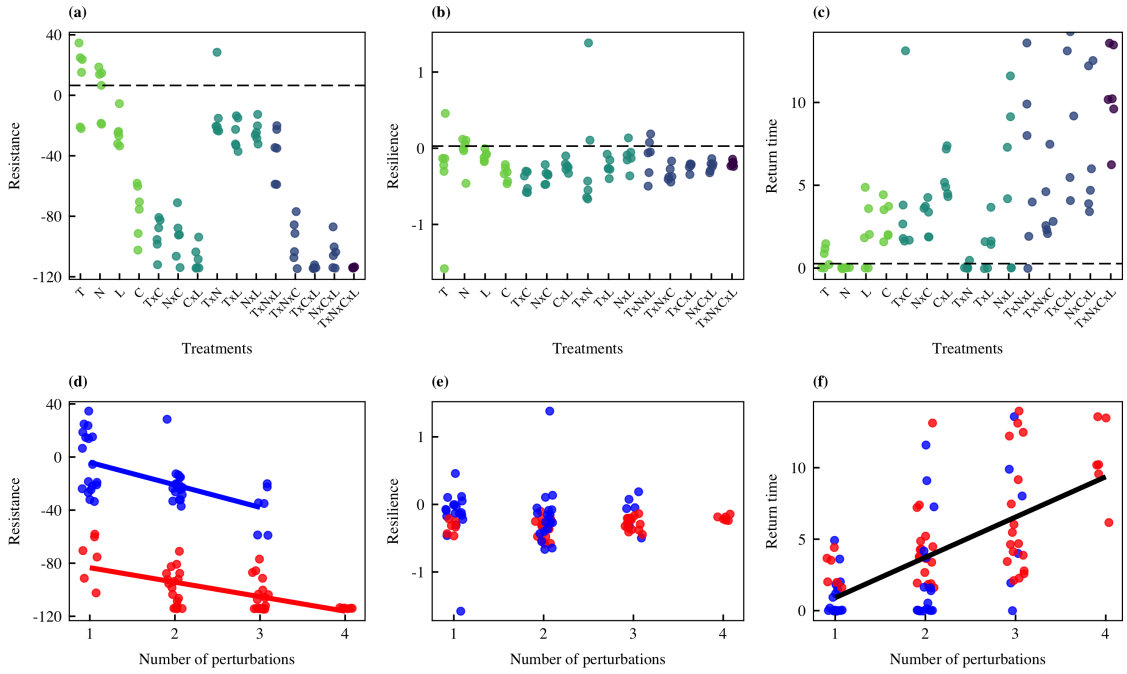


*Figure 3: Upper panels: observations of each disturbance combination for resistance (a), resilience (b) and return time (c). The dashed lines represent the mean of the control treatment. The colours represent the number of disturbances(s) (as in Figure 2). Lower panels: relationships between the responses and the number of perturbations (d-e-f). The colours represent the presence (red) and absence (blue) of the dominant driver (i.e., carbon enrichment perturbation "C"). Regressions represent the best model describing the relationship (comparison between linear and quadratic).*

Resistance was lowest in the carbon enrichment perturbation (C), with saturation decreasing to around 17% within two days of the press perturbation (Figure 2, 3a, Table 2, mean effect: -82.9% oxygen saturation with 95% CI=[-99.5, -66.3]). Light availability had a relatively smaller negative short-term effects on DO (Figure 2, 3a, with mean effects of -30.9% oxygen saturation (95% CI=[-47.5, -14.2]) and nutrients with an even smaller effect size with 95% confidence interval including zero: mean = -3.8% oxygen saturation (95% CI=[-20.3, 12.7])). In contrast, temperature had a small positive short-term effect on DO (Figure 3a, 2.7% oxygen saturation (with 95% CI=[-13.8, 19.2])). There were no significant interactions between the four disturbances affecting the short-term response to DO (Table 2).

Resilience, showing the medium-term response, was mainly negative, meaning that DO was returning towards initial levels (Figure 2, 3b). Resilience did not differ among treatments except for carbon enrichment (Table 2, Figure 3b), which caused a faster rate of recovery (more negative values) (Table 2, -0.36% oxygen saturation per day with 95% CI=[-0.72, -0.01]).

Observed return time (Figure 3c), showing the long-term response, was analysed with survival analysis (survival curves shown in Supplement Figure S1). An increase in the shape parameter corresponded to delayed recovery. Return time did not differ for disturbances applied independently (Table 2), but some positive, two-way interactions were significant (T:C and N:L) increasing recovery time.

*Table 2: Analysis of variance (type III, for resistance and resilience) and analysis of deviance (type III, for return time) of four-way linear model on the full dataset. Bold values indicated significant effects ($P < 0.05$).*

| | **Resistance** | | | | **Resilience** | | | | **Return time** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F value | Pr(>F) | Sum Sq | Df | F value | Pr(>F) | LR Chisq | Df | Pr(>Chisq) |
| (Intercept) | 255.8 | 1 | 1.199 | 0.277 | 4.349 | 1 | 43.847 | **<0.001** | | | |
| Temperature (T) | 22.0 | 1 | 0.103 | 0.749 | 0.155 | 1 | 1.562 | 0.215 | 0.099 | 1 | 0.753 |
| Nutrient (N) | 43.8 | 1 | 0.205 | 0.652 | 0.049 | 1 | 0.497 | 0.483 | 0.328 | 1 | 0.567 |
| Carbon enrichment (C) | 20592.5 | 1 | 96.533 | **<0.001** | 0.695 | 1 | 7.011 | **<0.01** | 3.405 | 1 | 0.065 |
| Light (L) | 2873.5 | 1 | 13.470 | **<0.001** | 0.082 | 1 | 0.823 | 0.365 | 1.188 | 1 | 0.276 |
| T:N | 480.1 | 1 | 2.251 | 0.138 | 0.117 | 1 | 1.177 | 0.281 | 0.461 | 1 | 0.497 |
| T:C | 552.8 | 1 | 2.592 | 0.111 | 0.099 | 1 | 1.001 | 0.320 | 5.498 | 1 | **0.019** |
| N:C | 286.6 | 1 | 1.343 | 0.250 | 0.006 | 1 | 0.058 | 0.811 | 0.417 | 1 | 0.518 |
| T:L | 22.6 | 1 | 0.106 | 0.746 | 0.062 | 1 | 0.623 | 0.432 | 0.383 | 1 | 0.536 |
| N:L | 26.2 | 1 | 0.123 | 0.727 | 0.000 | 1 | 0.002 | 0.963 | 11.533 | 1 | **<0.001** |
| C:L | 0.9 | 1 | 0.004 | 0.949 | 0.168 | 1 | 1.689 | 0.198 | 0.876 | 1 | 0.349 |
| T:N:C | 758.5 | 1 | 3.556 | 0.063 | 0.016 | 1 | 0.165 | 0.686 | 0.843 | 1 | 0.359 |
| T:N:L | 17.2 | 1 | 0.081 | 0.596 | 0.015 | 1 | 0.152 | 0.698 | 1.480 | 1 | 0.224 |
| T:C:L | 156.0 | 1 | 0.731 | 0.794 | 0.015 | 1 | 0.155 | 0.695 | 0.116 | 1 | 0.734 |
| N:C:L | 224.1 | 1 | 1.051 | 0.612 | 0.000 | 1 | 0.004 | 0.948 | 2.599 | 1 | 0.107 |
| T:N:C:L | 189.8 | 1 | 0.890 | 0.348 | 0.000 | 1 | 0.004 | 0.952 | 0.561 | 1 | 0.454 |
| Residuals | 16852.5 | 79 | NA | NA | 7.835 | 79 | NA | NA | - | - | - |

The relationship between response of dissolved oxygen and the number of perturbations was linear for resistance (quadratic term: $F_{1,12} = 0.03$, $P = 0.876$; linear term: $F_{1,12}= 6.14$, $P = 0.029$) and return time (quadratic term: $F_{1,11.8} = 2.76$, $P = 0.123$; linear term: $F_{1,11.8} = 17.81$, $P < 0.01$) but not for resilience (Figure 3e, quadratic term: $F_{1,12} = 3.05$, $P = 0.106$; linear term: $F_{1.12} = 0.86$, $P = 0.372$). Only the presence of the dominant disturbance (i.e., carbon enrichment) in the treatment affected resilience (dominant disturbance effect: $F_{1.85} = 6.7$, $P = 0.011$; number of perturbations: $F_{1,85} = 0.91$, $P = 0.343$; interaction: $F_{1.85} = 1.68$, $P = 0.198$). In contrast, the number of perturbations as well as the presence of the dominant disturbance (i.e., carbon enrichment) had a significant effect on resistance of DO (Figure 3d, dominant disturbance effect: $F_{1,11} = 155.02$, $P < 0.001$; number of perturbations: $F_{1,10.9} = 85.23$, $P < 0.001$; interaction: $F_{1,11} = 0.44$, $P = 0.520$). The number of perturbations only affected the return time (Figure 3f, dominant disturbance effect: $F_{1,10.9} = 2.11$, $P = 0.174$; number of perturbations: $F_{1,10.8} = 16.66$, $P < 0.005$; interaction: $F_{1,10.9} = 0.73$, $P = 0.410$). Overall, increasing the number of perturbations decreased resistance of DO and increased return time linearly, whereas it did not affect resilience (Figure 3d-e-f). The time required to recover increased by about two days per additional perturbation. And for comparison, the carbon enrichment treatment decreased the amount of oxygen by an average of 82.9%, while one additional perturbation caused, on average, a decrease of about 15% DO.

We compared predictability among the three temporal scales (Figure 4). Predictability was higher for resistance (adjusted $R^2$ always above 0.5) than it was for resilience and return time (adjusted $R^2$ below 0.5). The 95% confidence intervals of predictive power overlapped for all hypotheses, suggesting that no model performed significantly better for any response. Nevertheless, variation among models was still observed. Including all the interactions among environmental changes explained almost 90% of the variation observed in resistance (median of 88%). The dominant model,

despite that it uses, arguably, the lowest number of predictors, already explained 84% of variation. The 95% confidence intervals of resilience included zero for all hypotheses tested. For the return time, while its predictive power did not differ among the hypotheses, the 95% confidence interval of the dominant and additive hypotheses included zero. Including interactions to make predictions increased the predictive power up to 37%.

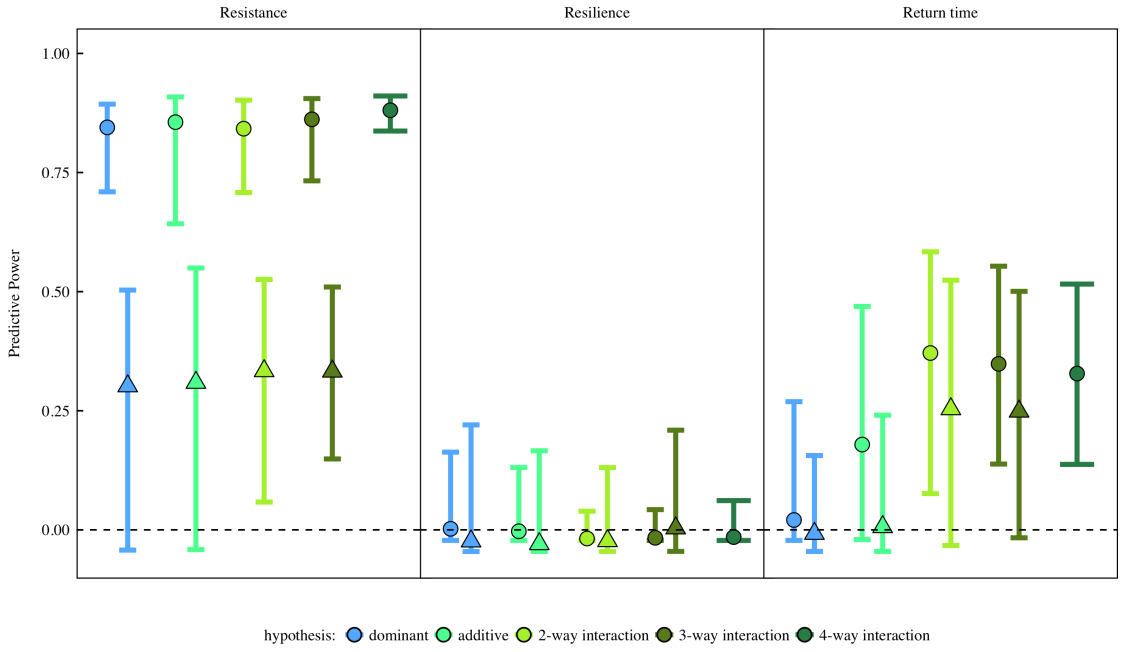


*Figure 4: Predictive power (adjusted $R^2$) of different models for resistance, resilience and return time. Median and 95% confidence intervals are shown. Circles show data for the whole experiment, triangles show data when the carbon enrichment treatment is excluded.*

Analyses that excluded the carbon enrichment treatment had generally lower predictive power, though the ranking of the various models remained similar (Figure 4). For example, the dominance and interactive hypothesis had similar accuracy for resistance, and including interactions increased predictive power for return time.

# Discussion

There is widespread concern that negative effects of global environmental change on aquatic systems will be exacerbated by interactions among multiple environmental changes (Darling & Côté 2008; Côté *et al.* 2016). We found scale dependent importance of interactions between disturbances on dissolved oxygen dynamics. The dominance model (i.e., when the disturbance with the largest effect is used to predict the combined effects of multiple disturbances) was a more parsimonious description of short-term response (i.e. resistance) than the interactive model, and the dominance model was similarly supported in the absence of the large effect of carbon enrichment on DO availability (Figure 4). There was little apparent effect of disturbances in the medium-term (i.e. for resilience), and interactions were more important in the long-term (i.e. for return time). The predictability of the short-term response was almost 90%, was around 0% in the medium-term, and about 40% for long-term response. Our results highlight that importance of interactions may be temporal scale dependent and that models of multiple environmental changes need to account for interactions when making longer term, but not for shorter term predictions. This result aligns with Christensen and collaborators' experiment (2006) in which they found that interactions between three environmental changes (temperature, drought and acidification) were stronger and synergistic at the end of their experiment due to stimulated total zooplankton biomass. Future studies should examine if these results hold for other ecosystem variables and for population dynamics and community structure.

Carbon enrichment had the greatest effect on dissolved oxygen dynamics, reducing resistance, increasing return time, while accelerating recovery from perturbations. The short-term negative effect on DO was caused by increase in bacterial per capita respiration and abundance (Figure 5), with little or no change in photosynthesis, which is often observed in natural systems (Amon & Benner 1996; Findlay *et al.* 2003). Indeed, half of the variance in DO is explained by the total bacteria density, reflecting the

importance of bacterial abundance and respiration for dissolved oxygen concentration (Figure 5).

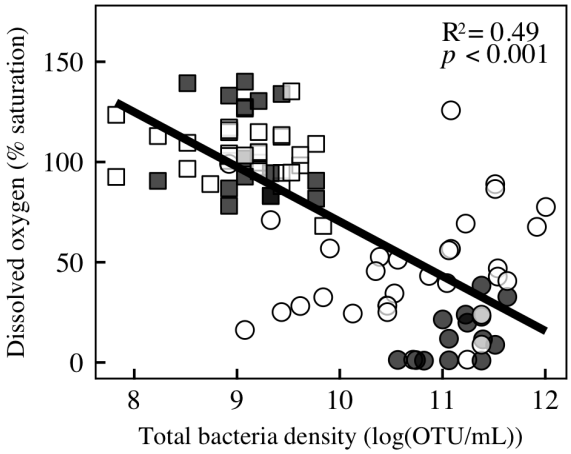


*Figure 5: Relationship between dissolved oxygen measurements and total bacteria densities for the three days following the disturbance(s). Symbols represent microcosms with (circle) or without (square) the carbon enrichment treatment. The dark symbols highlight the bacterial abundances estimated the day of maximum amount of change (i.e. resistance).*

It is interesting to note that this lack of resistance results from the ability of the biological community to quickly respond to the increased carbon available; such quick responses may be desirable in some situations (e.g. population recovery from low abundance) such that lack of resistance may sometime be a desirable property. Depletion of available carbon and subsequent reduction of bacteria (attributable to ciliate and rotifer consumption) could cause oxygen concentration to return to pre-perturbation levels. Reduced light availability had the same directional effect as carbon enrichment, although smaller in magnitude, and was likely attributable to a different underlying process - reduced light availability may have reduced photosynthesis of existing algae and also reduced algal growth and thus slowed oxygen production (Brennan & Collins 2015). It should be noted that the time scale of the response (e.g. maximum effect within three days of the disturbance) might be different for other response variables; the response of longer lived organisms than bacteria would likely take longer. Un-

derstanding how disturbances affected community composition and structure may be important to pinpoint the mechanisms underlying the observed responses. Our study is currently limited by lacking the population dynamic data for all species. As soon as this data get available, we will use a set of more mechanistic models to understand the dynamics of DO and its different predictabilities.

We also found that increasing the number of perturbations decreased resistance and increased recovery time, but did not affect resilience. This effect may be explained by the greater chance that the dominant disturbance would be present when the number of perturbations increases (Brennan & Collins 2015). Interestingly, for resistance, we also observed the detrimental effect of the number of perturbations in the absence of the dominant disturbance (Figure 3d). While two environmental changes (temperature and nutrient) did not have significant effects (Table 2), both disturbances had an effect in the presence of at least one other disturbance.

Previous conceptual work (Brook *et al.* 2008) and modelling studies (Sala *et al.* 2000) highlighted how the effects of environmental changes may be stronger than expected due to positive interactions and synergies between global change disturbances. We found limited evidence for positive interactions in our controlled experiment manipulating four common environmental changes of aquatic systems. Instead, the large effect of carbon enrichment relative to other disturbances resulted in the dominance model explaining the data best. This result conflicts with the notion of the widespread occurrences of positive interactions (Brook *et al.* 2008), but is in line with recent meta-analyses (Darling & Côté 2008; Jackson *et al.* 2016) and experiments (Brennan & Collins 2015). Darling and colleagues studied the presence of interactions on animal mortality in 112 factorial experiments and found truly synergistic effects in only one third of the reviewed studies, which was consistent across different disturbance types, as well as organisms and life stages considered. Moreover, Brennan and Collins (2015) looked at the growth response of a common freshwater algae under up to 8 different

types of environmental changes and found that the dominance model explained the data better than any additive or multiplicative model.

Findings can be influenced by experimental design choices. We had only two levels of each treatment (i.e., lower and higher temperature), as a result of choosing a relatively large number of environmental changes. This limited the type of interaction the experiment could reveal. It was unable to detect non-linear effects, nor how such non-linearity could be affected by other disturbances. That is, the experimental design could not evaluate if interactions among disturbances were state dependent. To do this would have required continuous manipulations of multiple disturbances to construct a disturbance-effect surface. Examining effects of continuous variation in multiple disturbances should be a priority for future research. Our findings may have also been influenced by our choice of treatment levels; for example, if we had chosen a much smaller carbon enrichment treatment, we may have found less support for the dominance hypothesis, though when we excluded this treatment from our analyses, the relative importance of the models, and their scale dependence, changed little.

To date, very few ecological studies of multiple environmental changes have attempted to predict responses across time (Petchey *et al.* 2015). We show that the immediate response (resistance) was very well predicted with few assumptions and data (dominance hypothesis). The additive hypothesis is commonly used to predict environmental changes (Crain *et al.* 2008), but here we showed that the dominance hypothesis, which estimated the same number of parameters and required a smaller experimental design, predicted the immediate effect of environmental changes very well. In contrast to resistance, resilience and return time were less predictable due to increasing variability in return time among replicates (Figure 3c) and small or no effect on the resilience (Table 2). The predictability of return time was somewhat improved by incorporation of interaction terms. Interactions could significantly affect species, but due to species co-tolerance (Vinebrooke *et al.* 2004) or functional redundancy (Fonseca

& Ganade 2001), ecosystem functioning may not be subjected to interactions between multiple environmental changes. Low predictability of resilience in response to the four environmental changes may have been caused by the process underlying recovery. Recovery likely resulted from arrested bacterial growth and consumption of bacteria by ciliates and rotifers. If we assume that none of these four disturbances increased the predation rate, there would have been no effect on recovery rate, though predation should at least have been higher in the increased temperature treatment level, and thereby increasing recovery rate (Pellan *et al.* 2016).

How the findings of any individual experiment performed with a specific community at a particular spatial scale apply at larger spatial scales and for different communities is an open question that will require considerable ingenuity to address. Gradual accumulation of individual experiments eventually provides opportunities for meta-analyses of such issues, but such accumulation can take a long time and is usually not part of a strategic / directed research effort. A preferable option is for multiple labs to coordinate to perform a carefully planned collection of individual experiments, which can then be analyses in combination. A single all-encompassing experiment would manipulate multiple environmental drivers (as we did) and include manipulations of spatial scale and community complexity. As mentioned above, such an experiment would also involve gradients (rather than discrete levels) of environmental disturbance. This experiment would require unusually large amounts of resources (time, space funding, personnel), though is not impossible to envisage. Finally, rigorous combining of findings from experiments and observational studies is a promising approach, and may be facilitated using process-based models and appropriate statistical methods of parameter inference (e.g. Bayesian methods).

The consequences of global environmental change on ecosystem stability are difficult to foresee, despite the urgent need for accurate predictions and recommendations to policy makers. Positive interactions have the potential to hamper such predictions,

however, they may be less widespread than suspected. Our results hence support the statement of Darling and Côté (2008) that the "prevailing ecological paradigm of synergies are rampant" may be overstated. Instead, we documented that the most parsimonious model for a microbial aquatic experiment showed scale dependence. Understanding what can be predicted and what cannot, and how this depends on temporal scale, is a challenge for future studies in order to provide accurate tools for ecosystem management.

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# Supplement

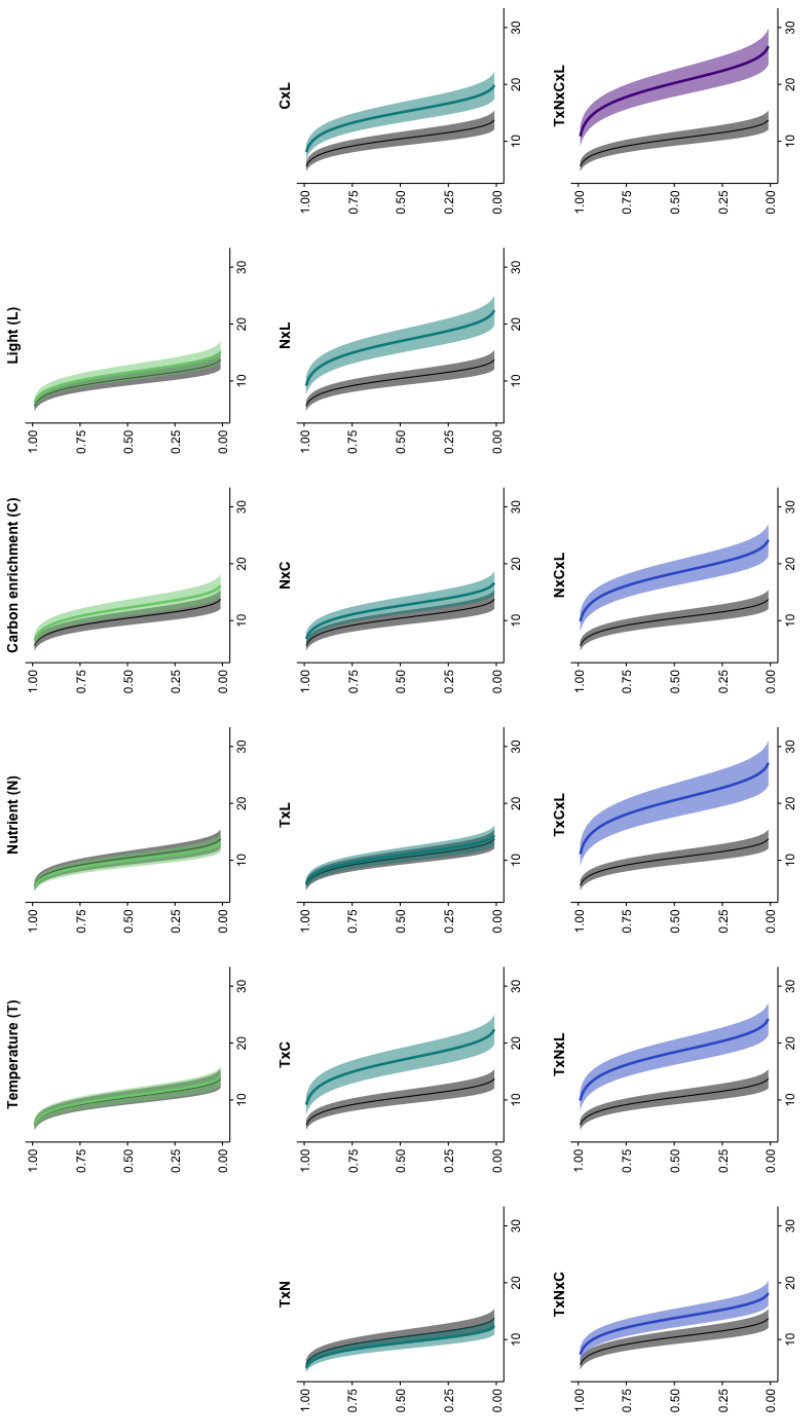


*Figure S1: Survival curves with a Weibull distribution of the return time. The hazard function depends on the scale ($\lambda$) and the shape ($p$) according the equation $h(t) = \lambda.p.t^{\,p-1}$. Colours indicate the number of combined perturbations (black = control, green = 1 perturbation, turquoise = 2, blue = 3, purple = 4).*

*Table S1: Analysis of variance (type II, for resistance and resilience) and analysis of deviance (type II, for return time) of four-way linear model on the full dataset. Bold values indicated significant effects ($P < 0.05$).*

| | Resistance | | | | Resilience | | | | Return time | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F value | Pr(>F) | Sum Sq | Df | F value | Pr(>F) | LR Chisq | Df | Pr(>Chisq) |
| Temperature (T) | 1454 | 1 | 6.818 | 0.011 | 0.158 | 1 | 1.594 | 0.21 | 6.192 | 1 | 0.013 |
| Nutrient (N) | 1170 | 1 | 5.484 | 0.022 | 0.049 | 1 | 0.497 | 0.483 | 2.926 | 1 | 0.0872 |
| Carbon enrichment (C) | 178085 | 1 | 834.818 | <0.001 | 0.712 | 1 | 7.174 | <0.01 | 39.253 | 1 | <0.001 |
| Light (L) | 14694 | 1 | 68.88 | <0.001 | 0.078 | 1 | 0.785 | 0.378 | 40.564 | 1 | <0.001 |
| T:N | 165 | 1 | 0.771 | 0.383 | 0.119 | 1 | 1.196 | 0.277 | 2.162 | 1 | 0.142 |
| T:C | 16 | 1 | 0.073 | 0.788 | 0.099 | 1 | 1.003 | 0.32 | 8.792 | 1 | <0.01 |
| N:C | 147 | 1 | 0.687 | 0.41 | 0.005 | 1 | 0.055 | 0.815 | 5.642 | 1 | 0.018 |
| T:L | 0 | 1 | 0 | 0.996 | 0.062 | 1 | 0.624 | 0.432 | 0.063 | 1 | 0.802 |
| N:L | 550 | 1 | 2.581 | 0.112 | 0 | 1 | 0.002 | 0.967 | 21.984 | 1 | <0.001 |
| C:L | 531 | 1 | 2.489 | 0.119 | 0.169 | 1 | 1.701 | 0.196 | 0.345 | 1 | 0.557 |
| T:N:C | 630 | 1 | 2.954 | 0.09 | 0.016 | 1 | 0.166 | 0.685 | 3.784 | 1 | 0.052 |
| T:N:L | 60 | 1 | 0.283 | 0.596 | 0.015 | 1 | 0.153 | 0.697 | 0.939 | 1 | 0.332 |
| T:C:L | 15 | 1 | 0.069 | 0.794 | 0.015 | 1 | 0.154 | 0.696 | 0.076 | 1 | 0.783 |
| N:C:L | 55 | 1 | 0.259 | 0.612 | 0 | 1 | 0.004 | 0.947 | 8.414 | 1 | <0.01 |
| T:N:C:L | 190 | 1 | 0.89 | 0.348 | 0 | 1 | 0.004 | 0.952 | 0.561 | 1 | 0.454 |
| Residuals | 16852 | 79 | NA | NA | 7.835 | 79 | NA | NA | - | - | - |

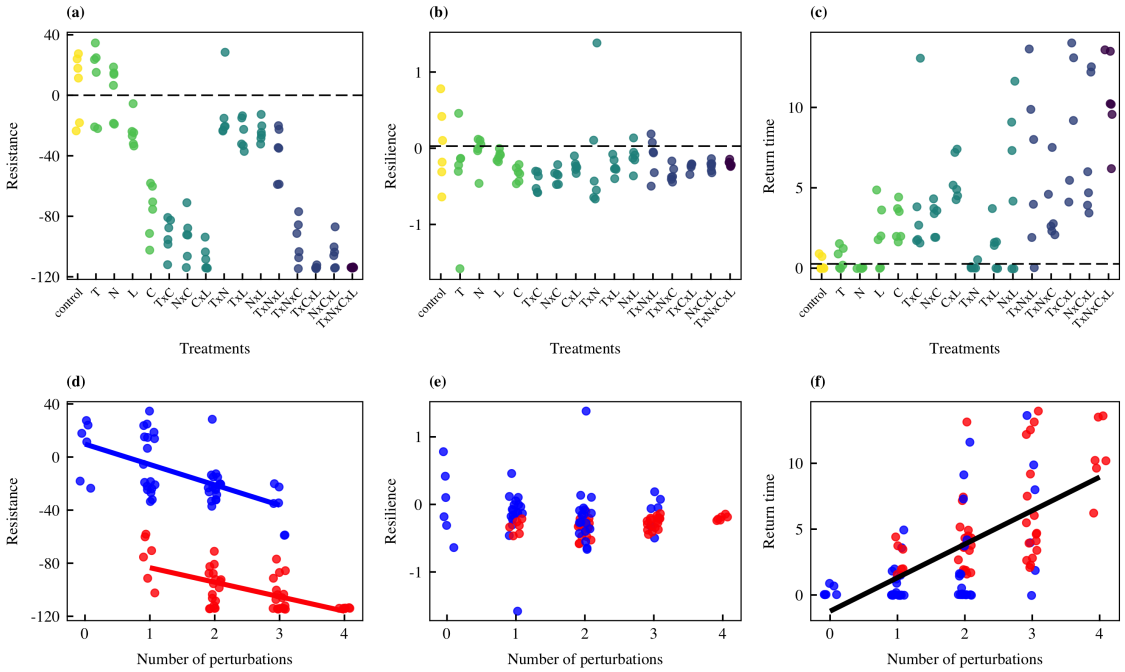


*Figure S2: Upper panels: observations of each disturbance combination for resistance (a), resilience (b) and return time (c). The dashed lines represent the mean of the control treatment. The colours represent the number of disturbances(s) (as in Figure 2). Lower panels: relationships between the responses and the number of perturbations (d-e-f). The colours represent the presence (red) and absence (blue) of the dominant driver (i.e., carbon enrichment perturbation "C"). Regressions represent the best model describing the relationship (comparison between linear and quadratic). Values for control treatments are included to show their variability and to validate the methods (the expectation is that the control values are centered on zero).*

# Chapter 3

# Multiple environmental disturbances: from individuals to ecosystems functions.

**Abstract**

Freshwater ecosystems are considered as some of the most threatened ecosystems due to their proximity to human populations and the increasing anthropogenic perturbations. Environmental disturbances affect systems at all levels of ecological organisation. To make things worse, environmental disturbances are more likely to occur simultaneously and interact between each other, raising the possibility of ecological surprises that are difficult to predict. Whereas policymakers and managers require a high level of predictability of environmental change impacts on ecosystems, very few studies examined multiple environmental disturbances (with more than two disturbances) or examined impacts across levels of organisation. Additionally, none - so far - had investigated these both aspects for prediction of multiple environmental disturbances across levels of organisation.

I used a microcosm experiment with a microbial community (bacteria, algae, ciliates and rotifer) to test the prevalence, direction and strength of interactions between four common environmental disturbances (increasing temperature, increasing organic matter, decreasing N:P ratio, and decreasing light availability). I analysed temporal changes of individual traits, population dynamics, community structure and composition and ecosystem functions using a general additive model that took into account the temporal change with a spline function and estimated the main effects and the 2-, 3- and 4-way interactions. To compare the disturbances across levels of organisation, I used a correlation between levels of organisation that described the similarity of the direction of the response to multiple environmental disturbances (main effects and interaction terms).

I highlighted that 1) the four environmental disturbances affected all levels of organisation differently. 2) The interactions were relatively rare but complex (both synergistic and antagonistic interactions were present). Therefore, selecting one scenario of interacting environmental drivers may not be the optimal solution. Rather, future research should focus on the relationship between the ecosystem function (i.e., response variable of interest) and the number of environmental drivers. 3) The presence of similarity of response to disturbances within population dynamics was evidence of bottom-up consequence on the food web. And 4) the presence of similarity of response between levels of organisation could improve models to up-scale multiple environmental disturbances through levels of organisation.

# Introduction

Being at the interface between marine and terrestrial ecosystems, freshwater systems are considered to be the most threatened ecosystem type due to increasing anthropogenic perturbations (Dudgeon *et al.* 2006; Sala *et al.* 2000) while they supply numerous goods and services (e.g., drinking water, transport, irrigation, recreation and fisheries; Millenium Ecosystem Assessment *et al.* 2005). Freshwater ecosystems are mostly impacted by habitat degradation, pollution, flow regulation, water extraction, fisheries over-exploitation, alien species introductions and climate change (e.g., Strayer & Dudgeon 2010; Woodward *et al.* 2010).

Environmental disturbances affect individual performance, populations size, community structure and ecosystems functions. An important issue is the lack of studies that measured consequences of environmental change at different levels of organisation (but see reviews of Simon & Townsend 2003 on invasive species and Woodward *et al.* 2010 on climate change). Already, up-scaling processes to describe spatial, temporal or organisational patterns begets problems to ecologists (Levin 1992).

In this research, I focused on effects of four common environmental disturbances in freshwater ecosystems: increasing temperature, increasing organic pollution, increasing inorganic pollution (decreased N:P ratio) and decreasing light availability. The last three disturbances would impact the basal resources (primary producers and detritus) on which aquatic ecosystem structure and functioning are based (Allan & Castillo 2007). While increasing temperature would impact differently each level of organisation (Woodward *et al.* 2010).

From terrestrial ecosystems, inputs of organic matter provide energy to stream and riverine ecosystems (Kominoski & Rosemond 2012). Organic matter quality and quantity and biological processing are altered with climate change (e.g., elevated atmospheric CO2, changes in precipitation and hydrology, increased temperature), land use

change (e.g., reductions and shifts in watershed vegetation, agriculture and mining) and biodiversity loss (e.g., loss of conifer trees, increases in N-fixing and plantation species, and reductions in genetic variation) (Kominoski & Rosemond 2012). Increasing organic matter input enhances bacterial growth (Baines & Pace 1991) and thus favours heterotrophic ecosystems (e.g., Cole *et al.* 2000; Duarte & Prairie 2005).

With intensive agriculture and its use of fertilizers, infiltration and runoff of non-point source pollution (i.e., inorganic pollution) affect aquatic ecosystems (Carpenter *et al.* 1998). Indeed, inorganic nutrients (mainly nitrogen and phosphorus) promote toxic algal bloom, loss of oxygen, fish kills, loss of biodiversity, loss of aquatic beds and coral reefs (Carpenter *et al.* 1998). These consecutive consequences of eutrophication on ecosystems are the cause of nonlinearity and hysteresis observed in shallow lakes, coral reefs and oceans (Scheffer *et al.* 2001).

With increasing organic and inorganic pollution, changes in community structure affect the ecosystem (i.e., eutrophication) and can enhance phytoplankton blooms and suspended particles which increase the water turbidity. Water turbidity affects the structure and functions of lake ecosystems by reducing the light availability and therefore the growth of benthic plants which can act as refuges for zooplankton (actor of a top-down control to favour water clarity; Scheffer & Nes 2007). Therefore, these three disturbances (organic matter, nutrient composition, and light) affect the whole food web due to bottom-up consequences (e.g., Wallace *et al.* 1997).

On the other hand, increasing temperature affects different components of ecological systems and consequences can be either bottom-up or top-down. At the organism level, increasing temperature increases the metabolic rate of individuals, which reduces the body size and enhances the growth rate (Brown *et al.* 2004). Temperature is also a key factor that impacts inter-specific interactions and can lead to the loss of top predator (e.g., Kordas *et al.* 2011; Petchey *et al.* 1999; Harley 2011). With increasing temperature, populations extend their distribution range (Rahel & Olden 2008)

and thus might alter community composition and structure due to new inter-specific interactions (Le Roux & McGeoch 2008) and the ecosystem functions (Petchey *et al.* 1999). The effect of temperature is a well-understood example of scaling across levels of organisation. Three theories emerged and provided insights to understand and predict consequences of warming on natural communities (Woodward *et al.* 2010). The metabolic theory, the foraging theory and the ecological stoichiometry. Where body size is a key variable to up-scale across levels of organisation for the metabolic and the foraging theories, while the ecological stoichiometry theory is based on the transfer along levels of organisation of C:N:P ratio (Woodward *et al.* 2010).

To make things worse, it is more likely that perturbations act simultaneously rather than in isolation (Brook *et al.* 2008). A recent study highlighted that aquatic ecosystems were more likely to undergo three perturbations simultaneously (Schäfer *et al.* 2016). Because these four disturbances act differently on biological organisations, I would expect ecological surprises when the disturbances are combined due to the presence of interactions, leading to uncertainties in the predictability of global change (Christensen *et al.* 2006; Doak *et al.* 2008; Sala *et al.* 2000; Darling & Côté 2008).

Synergistic and antagonistic interactions are defined in comparison to the additive hypothesis (i.e., the sum of environmental disturbances' effects acting in isolation; see Figure 2 in Chapter 1). For instance, when the total effect of two disturbances is greater than the additive hypothesis, the interaction is called synergistic. Whereas, when the total effect is smaller than the additive hypothesis, the interaction is called antagonistic. The determination of antagonistic and synergistic is well defined when two environmental disturbances are combined and acting in the same direction (both positive or negative effects) (Folt *et al.* 1999; Piggott *et al.* 2015). However, the definition of interaction gets more difficult when more than three disturbances are combined (e.g., Tekin *et al.* 2016). When interactions are observed, the up-scaling of the effects of disturbances and their interactions across biological organisation become more

challenging.

The aim of this research was to investigate the consequences of multiple environmental disturbances across four levels of biological organisations (individual traits, population dynamics, community structure and composition, and ecosystem functions), and the implication of the frequency, direction and strength of interactions between these four environmental disturbances. According to a recent meta-analysis on the interactions between two environmental disturbance, I expected that interactions between multiple environmental disturbances would be mainly antagonistic (Jackson *et al.* 2016).

# Material and Methods

## Experimental system

I used the same experimental system presented in Chapter 2 "Temporal scale dependent interactions between multiple environmental disturbances in microcosm ecosystems." To summarise the experimental design, I initiated the community in 100 mL of protozoan pellet medium at 0.28 $g.L^{-1}$ with two wheat seeds to provide a slow nutrient release. The community included two species of bacteria, four species of algae, 12 species of ciliates and one species of rotifer. And among the ciliates, three species were predators.

For one week, all microcosms were at control levels for the establishment of the community. On the eighth day, I applied four different disturbances: a temperature increase, a N:P ratio decrease (with phosphorus addition), an organic matter increase and a decrease in light availability. These were applied in a four-way fully factorial design with six replicates in each of the sixteen treatment combinations.

## Data acquisition

The complexity of the system used required different methods to identify and estimate abundances of each species. I estimated bacterial abundance by plating diluted samples on agar plates, the algae with a flowCAM that took pictures, the small ciliates with videos and direct counting the larger ciliates (i.e., predators) with a microscope. For the ecosystem functions, I regularly acquired the biomass from the videos and the oxygen levels were measured at the end of the light (8 hours) and the dark (16 hours) periods.

While oxygen levels were measured every day, the abundance of each species was measured every three days. The sampling of the 96 microcosms was split into three parts, and in a way that two replicates of each treatment were sampled every day.

**Bacteria plating.** From the microcosm, I sampled 20 µL diluted in 180 µL of diluted Protozoan Pellet Medium (0.05 $g.L^{-1}$). I plated 8 µL at three different dilutions. Plates were incubated for 12 hours at 37°C and kept at 5°C until counting. I selected the dilution when more than 10 Colony Forming Units (CFU) were present and reported the number of CFU with its dilution factor.

**FlowCAM.** I estimated the algal densities using a FlowCAM with the following set up: a flow rate of 0.5mL/min, a magnification 4x, an efficiency of ∼71%, and the Auto Image mode which took a picture every 20ms. This setting resulted in processing ∼0.1mL for each sample. The recognition of algae was based on pictures and the software VisualSpreadsheet 3.4.8.

Monocultures were processed identically as the samples and provided the morphological traits (e.g., area, aspect ratio, circularity, elongation, length, width) to base the identification with randomForest classification (see Supplement for more detailed on the identification).

**Videos analysis.** I sampled 1 mL and placed it in a cell counting chamber (Sedgewick-Rafter S52 Pyser-SGI). I took the videos using a camera (Hamamatsu Dig-

ital camera C11440) attached to a microscope (Leica M205C 0.63X), and the software HCImageLive (version 4.0.6.3). I set the magnification at x2.5 which allowed recording ∼72 µL per video. For each sample of a microcosm, I took two videos of five seconds each with 25 frames per second (i.e., 125 frames with 10 ms exposure and 40 ms delay).

I processed the videos following Pennekamp *et al.* (2015) to extract morphological traits (e.g., area, shape, length and width) and behavioural traits (e.g., movement speed, net displacement and turning angle) with `BEMOVI` package. Based on these traits, I used a machine learning classifier to identify the species. I detailed the comparison of two classification methods (randomForest and support vector machine classification) in the Supplement. Given the results of the comparison, I used the support vector machine classifier which was less sensitive to unbalanced data information, and therefore allowed me to be able to identify rare species (i.e., predators).

The caveats of this method are 1) the requirement of movement, 2) high enough abundance for reliable detection, and 3) the morphological traits with large enough interspecific variation to identify the species accurately. To take into account these caveats, I counted bigger ciliates by eye under binocular. Indeed, regarding the reliable detection, to have a good estimation of abundance it is often required to count a minimum of 10 individuals. Given, the volume search with the videos (72 µL), a species should have an abundance of 140 individuals per mL to be reliable using this method. If the count observed is lower, the estimation might be either overestimated or underestimated.

**Manual counting.** Bigger ciliates and predators have generally lower abundance than their prey. Therefore, I manually counted seven species (*Rotifer* sp., *Blepharisma japonicum*, *Frontonia* sp., *Stentor coeruleus*, *Actinophrys sol*, *Dileptus anser* and *Paramecium bursaria*) either in 100 µL, 1 mL or 5 mL according to their abundance.

**Dissolved oxygen.** Twice a day, at the end of the light (16 hours) and the dark period (8 hours), I measured the dissolved oxygen saturation (% a.s.) using a

non-invasive method called chemical-optical sensor (Fibox 4 trace, PreSens, Germany; Altermatt *et al.* 2015). Note that 100% a.s. is equivalent to ∼21% O2.

## Response variables

**Morphological and behavioural traits.** For each species detected by videos, the body size was extracted from the videos. The body size was an extrapolation of the mean area of the individual (i.e., trajectory summary of a moving particle), and scaled from pixel to $\mu m^2$. With a 2.5x magnification, the conversion ratio pixel to micrometer was 1000/240.

From the movement of individuals, I extracted two behavioural traits (the net speed and the variation of turning angle). The net speed was the net displacement (distance between the first and the last coordinate of a trajectory) divided by the total duration of the trajectory. And the variation of turning angle (i.e., standard deviation) indicated how much and how often there was a change in the direction of the trajectory.

I based the analyses on population medians of these three individual traits (per species and per replicate). I could identify *Colpidium striatum*, *Tetrahymena thermophila*, *Rotifer* sp., and *Paramecium* (combining *P. caudatum* and *P. bursaria*). Some other species (*Blepharisma japonicum*, *Frontonia* sp., *Euplotes* (combining *Euplotes* sp. and *E. daidaleos*) were identified, but with very low density, the analysis of their traits was heavily influenced by sampling error. Additionally, and unfortunately, *Nassula aurea* was not identified possibly due to extinction or misidentification.

**Population dynamics.** I counted each species using different methods (plating, flowCAM, manual counting or automated counting) and obtained the densities following the different formulas in Table 1.

*Table 1: Formulas to obtain the density of each population using different methods.*

| Method | Density per mL |
|---|---|
| flowCAM (algae) | Count / volume processed |
| Plating (bacteria) | $CFU/8 * 1000 * 10^{dil.factor}$ |
| Manual counting | Count / volume sampled |
| Automated counting | Count * (1/0.144) |

**Richness.** The richness was the number of species present in the community.

**Simpson's diversity index (1-D).** This index takes into account the relative abundance of each species, and it estimates the probability that two individuals randomly selected from a sample will belong to different species (Table 2). An increase of this index (1-D) would relate to an increase in diversity. This index was estimated using the R package vegan (Jari Oksanen *et al.* 2017).

*Table 2: Formulas used to calculate diversity indices. $P_i$ is the proportion of individuals belonging to species i. Formulas from Simpson 1949.*

| Metric | Formula |
|---|---|
| Richness (S) | Number of species |
| Simpson's diversity (1-D) | $1 - \sum_{i=1}^{S} P_i^2$ |
| Connectance | $L/S^2$ |

**Connectance.** The connectance was estimated using the number of links between two species observed in the community (see Table **??**) and the number of species (Table 2). The connectance is a measure of the complexity of the food web and may be related to stability. A community with high connectance is expected to be more stable when facing perturbation (De Angelis 1975).

**Biomass.** The biomass (or biovolume) was estimated using the morphological traits from the videos analysis of all individuals detected (i.e., moving particles), not only the individuals with species identity. The measurement was the sum of the individual's biovolume (by fitting an ellipsoid using the length and width of an individual).

**Respiration and net primary production.** At the end of the light period (16 hours - with measurement in the morning), the % of air saturation (% a.s.) was the result of the net effect of photosynthesis and respiration (i.e., "$DO_{light}$"). Whereas, at the end of the dark period (8 hours - with measurement in the afternoon), the % a.s. reflected only the respiration of the community (i.e., "$DO_{dark}$"). From these two measurements, the respiration and net primary production (NPP) rates per hour were calculated as:

$$\text{Respiration}_t = \frac{\text{DO}_{dark_t} - \text{DO}_{light_t}}{8}$$
$$\text{NPP}_t = \frac{\text{DO}_{light_t} - \text{DO}_{dark_{t-1}}}{16} - \text{Respiration}_{t-1}$$

**Decomposition.** Before and at the end of the experiment, the wheat seeds were dried at 100°C for 10 hours and weighted. Then, the decomposition was measured as the absolute difference in weight of the seeds (used for a slow nutrient release) between the beginning and the end of the experiment.

## Statistical analyses

For each variable of each replicate at each time point, I estimated the $\log_{10}$ response ratio between the value in replicate microcosms and the mean of the control. The time series of this response ratio was analysed using a generalised additive model, with the treatments as the fixed predictors and a spline function of the time in interaction with the treatment using the R package `mgcv` (Wood 2011). Treatment main effects, two-way, three-way and four-way effects were estimated. Only one variable, the decomposition, consisted of one measurement per microcosm. Therefore, I analysed the effect with a linear model and an analysis of variance of type III.

To examine how treatment effects scale from individual traits to ecosystem functions, I estimated the effect size (Hedges'd) of the main effects and interaction terms (Hedges & Olkin 1985), using the following equation:

$$\mathrm{d} = \frac{\bar{Y_i} - \bar{Y_C}}{\sqrt{\frac{(\mathrm{N}_i - 1).s_i^2 + (\mathrm{N}_C - 1).s_C^2}{\mathrm{N}_i + \mathrm{N}_C - 2}}}$$

where $\bar{Y_i}$ was the mean estimate/interaction term of a treatment, $\mathrm{N}_i$ the number of replicates and $\mathrm{s}_i$ the standard deviation; $\bar{Y_C}$, the mean of the control, $\mathrm{N}_C$ the number of replicates and $\mathrm{s}_C$ the standard deviation.This method is largely used in meta-analyses to compare different studies that investigated different variables and species. Due to different sample size between studies, a parameter $J$ can be calculated to correct for small sample size. However, I did not correct the effect size as I was comparing main effects and interaction terms among different variables in my experiment (i.e., the same sample size for all treatments). To examine the similarity/dissimilarity of the response to disturbances within and between levels of organisation, I analysed the correlation of the Hedges'd (Pearson correlation using pairwise observation).

# Results

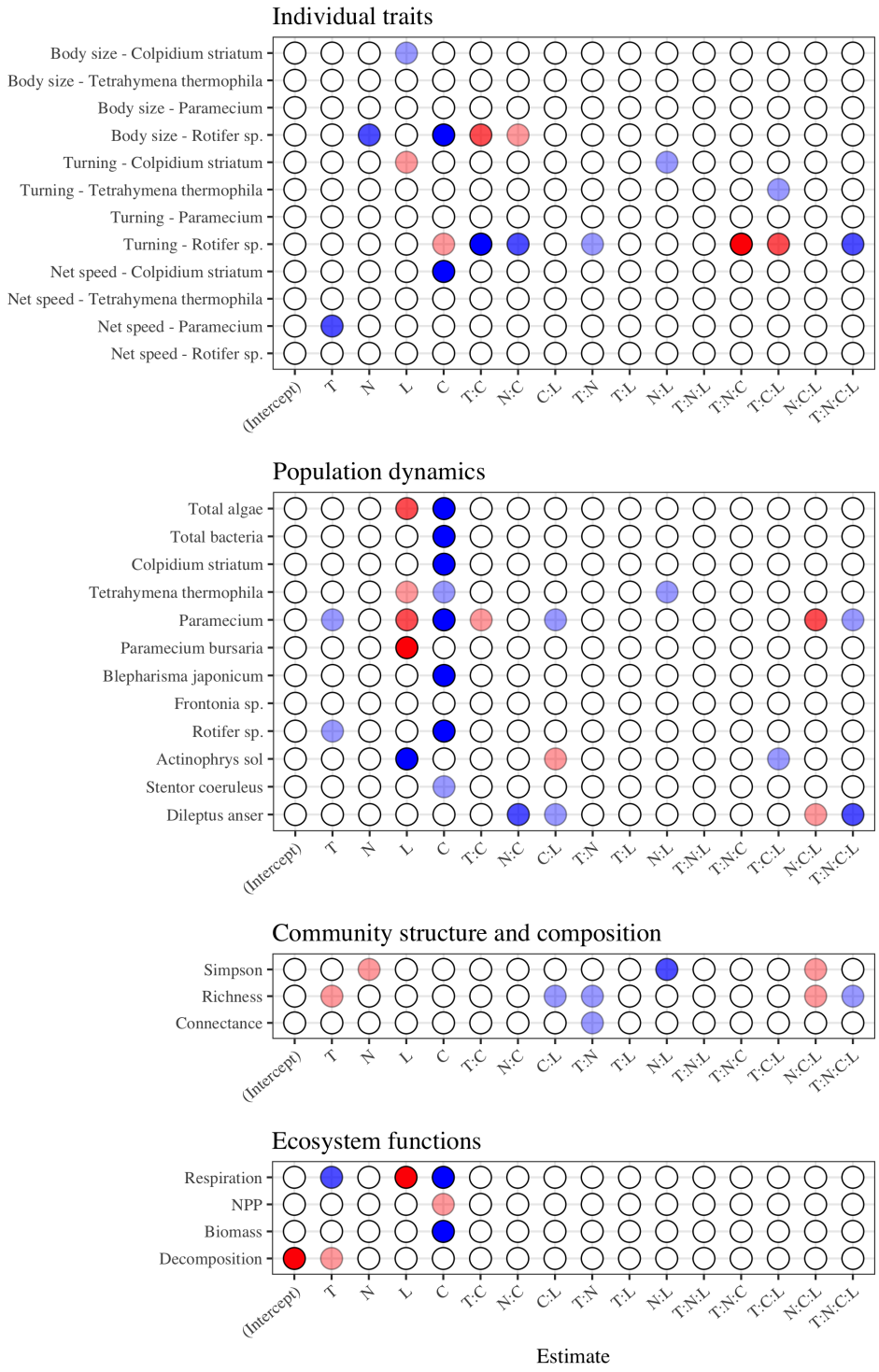


*Figure 1: Main effects and interactions terms of four environmental disturbances on individual traits, population dynamics, community structure and composition and ecosystem functions. Disturbances are indicated with the letters* ***T****: increased temperature,* ***N****: decreased N:P ratio,* ***L****: decreased light availability, and* ***C****: increased organic matter. The colours reveal significant effects or interactions terms with a P value < 0.05; red for a negative effect / interaction term and blue for a positive effect / interaction term. All time series and statistical analyses are in Supplement.*

## Individual traits

Overall the four environmental disturbances and their interactions did not consistently affect the individual traits of the species detected by videos (*Colpidium striatum*, *Tetrahymena thermophila*, *Paramecium* and *Rotifer* sp.). Interactions between the disturbances were rare, only ∼8% of the interactions were significant.

The body size of *Colpidium* increased when the light availability decreased ("L", F=6.557, $P$=0.011); and the body size of *Rotifer* increased with increased organic matter ("C", F=19.926, $P$<0.001) and decreased N:P ratio ("N", F=7.476, $P$=0.007). Antagonistic interactions were observed for *Rotifer* when organic matter ("C") interacted with either temperature ("T:C") or nutrient ("N:C"). The body sizes of *Paramecium* and *Tetrahymena* were not affected by any disturbances in isolation or in combination (all $P$>0.05).

The net speed was affected only by disturbances in isolation. The net speed increased for *Colpidium* with organic matter enrichment ("C", F=17.662, $P$<0.001); and for *Paramecium* with increased temperature ("T", F=7.789, $P$=0.005).

The turning angle decreased for *Rotifer* due to an increase in organic matter ("C", F=4.929, $P$=0.027) and for *Colpidium* due to a decrease in light availability ("L"; F=4.825, $P$=0.029). Interactions between two disturbances were antagonistic (from negative to positive effect) for *Rotifer* and *Colpidium*, therefore increasing the turning angle. The presence of opposing signs of interaction terms (negative for "T:N:C" and "T:C:L"; and positive for "T:N:C:L") for *Rotifer* indicated an overall dampening effect when increasing the number of disturbances. Surprisingly for *Tetrahymena*, which was not affected by the disturbance in isolation or between two disturbances, increased its foraging effort (i.e., turning) when temperature, organic matter and light disturbances ("T:L:C") were accumulated.

## Population dynamics

Overall, two disturbances in isolation (organic matter enrichment "C" and decrease light availability "L") had large effects on populations dynamics, while decreased N:P ratio did not affect any population dynamics. Organic matter enrichment increased the abundances of the resources (algae and bacteria), of the consumers except *Paramecium bursaria* and *Frontonia* sp., and of the omnivorous *Stentor coeruleus*.

For resources and consumers, a decrease in light availability had either a negative effect on the abundances (e.g., total algae, *Paramecium bursaria*) or no effect on the abundances (e.g., total bacteria, *Colpidium striatum*). The predator *Actinophrys sol* increased in abundances ("L", F=12.304, $P<0.001$).

Temperature increase had mainly no effect on population dynamics, except for *Paramecium* and *Rotifer* sp. that increased with temperature ("$T_{Paramecium}$", F= 4.156, $P$=0.042; "$T_{Rotifer}$", F=4.593, $P$=0.033).

Interactions between the disturbances were rare, only ∼8% of the interactions were significant. Mainly the two-way interactions were antagonistic for *Tetrahymena*, *Paramecium* and *Actinophrys*. Indeed, the densities of *Tetrahymena* increased when "N" and "L" were combined ("N:L", F=4.500, $P$=0.035), compared to the negative effect of decreased light availability ("L"). The densities of *Paramecium* decreased when organic matter ("C") interacted with temperature ("T:C", F=4.845, $P$=0.028), while the densities increased when "C" interacted with "L" ("C:L", F=4.856, $P$=0.028), resulting in reducing the negative effect of the light disturbance. The densities of *Actinophrys* reduced when "L" interacted with "C" ("C:L", F=5.246, $P$=0.022), compared to the increase observed when "L" acted in isolation. For *Dileptus*, I observed an increase in the densities when "C" interacted with "N" or "L" ("C:N", F=8.800, $P$=0.003; "C:L", F=6.498, $P$=0.011), while the disturbances in isolation did not affect the densities.

Interestingly, the 3- and 4-way interaction terms reversed the 2- and 3-way interaction term respectively. This pattern was observed for *Actinophrys*, *Paramecium* and

*Dileptus*.

## Community structure and composition

While the organic matter enrichment and the decrease in light availability had large effects on population dynamics, they did not affect community structure or composition. However, the interactions between the disturbances were more common with 21% of significant interactions.

The Simpson diversity index decreased (i.e., diversity decreased) with decreased N:P ratio ("N", F=5.115, $P$=0.024). But this negative effect was counteracted when "N" interacted with a decrease in light availability ("N:L", F=8.515 , $P$=0.004); and additionally, this antagonistic interaction was also reverse when organic matter was added to the previous two disturbances ("N:L:C", F=5.716, $P$=0.017).

The richness decreased with increased temperature ("T", F=5.939, $P$=0.015). Nevertheless, this negative was reversed when "T" interacted with "N" ("T:N", F=4.658, $P$=0.032). And a synergistic interaction was detected between "C" and "L" ("C:L", F=5.627, $P$=0.018), while none of these two disturbances affected the richness while in isolation. Interestingly, I observed the pattern of reverse sign between 3- and 4- way interaction terms again.

The connectance of the community was not affected by these four environmental disturbances. Only one positive interaction term between the temperature increase and decreased N:P ratio was found significant and therefore increasing the connectance ("T:N", F=3.935, $P$=0.048) while these two disturbances had no significant effect in isolation.

## Ecosystem functions

None of the interactions tested were significant for the four ecosystem functions. The decomposition increased with increasing temperature ("T", F=6.097, $P$=0.016), and

the biomass increased with organic matter enrichment ("C", F=38.001, $P$<0.001). The net primary production decreased with increasing organic matter enrichment ("C", F=-2.377, $P$=0.018). The respiration increased with temperature ("T", F=2.768, $P$=0.006) and organic matter enrichment ("C", F=4.169, $P$<0.001), whereas respiration decreased with the reduction of light availability ("L", F=-3.823, $P$<0.001).

## Overall effect sizes of main effect and interaction terms within and between levels of organisation

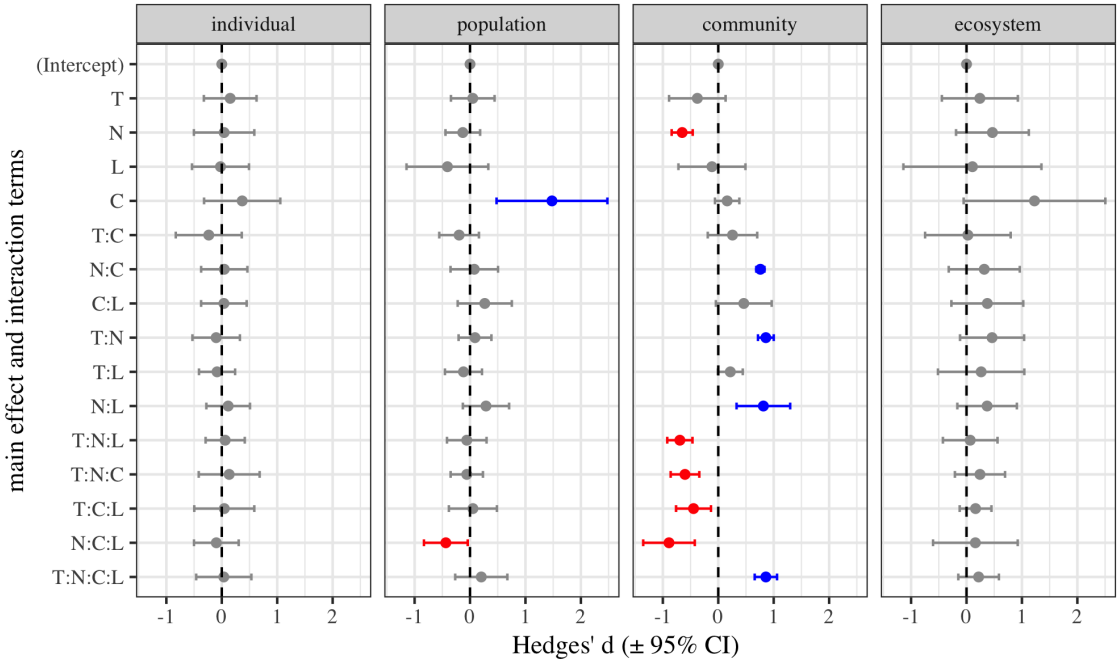


*Figure 2: Mean effect sizes of main effects and interaction terms within each level of organisation. The colours revealed significant average effect / interaction terms based on the 95% confidence interval. Red is negative, blue is positive, and grey is the absence of significant average effect / interaction term.*

For individual traits, the overall main effects and interaction terms were not significant and showed little variation within the three individual traits studied. Note that only

∼8% (10 significant interactions over 132 interactions tested) of the interactions tested were significant (Figure 1).

On population dynamics, most of the main effects and interaction terms were not significant, except for the organic matter enrichment ("C") that increased on average the populations densities, and for the 3-way antagonistic interaction "N:C:L" that decreased the population densities only for Paramecium and Dileptus (Figure 1). In contrast to the individual traits, the variation within the population dynamics is higher for the main effects. Note that only ∼8% (11/132) of the interactions tested were significant (Figure 1).

The three variables describing the community showed more consistent responses to disturbances (Figure 2). A decrease in N:P ratio ("N") decreased the community variables. As mentioned in the previous section describing each community variables, the pattern of opposing signs between the 2-, 3- and 4-way interaction terms is observed on the average interaction terms (Figure 2). This pattern is particularly revealed at the community level as 21% of the interaction tested were significant (7/33; Figure 1).

The average effects and interactions terms of the ecosystem functions were not consistent within ecosystem functions due to different directions of ecosystem responses to disturbances (for example, respiration and net primary production). None of the interactions tested were significant.

I observed the similarity of the response to the disturbances within variables describing each level of organisation (Figure 3) and between levels of organisation (Figure 4).

At the individual level (Figure 3a), the body size and the net speed responded similarly the perturbation. However, I could notice that this similarity resulted in the unique positive effect of organic matter enrichment. The dissimilarity between the changes in direction (i.e., turning) and body size or net speed is due to the negative effects of organic matter enrichment and reduced light availability on *Rotifer*'s and

*Colpidium*'s turning angles respectively (Figure 1).

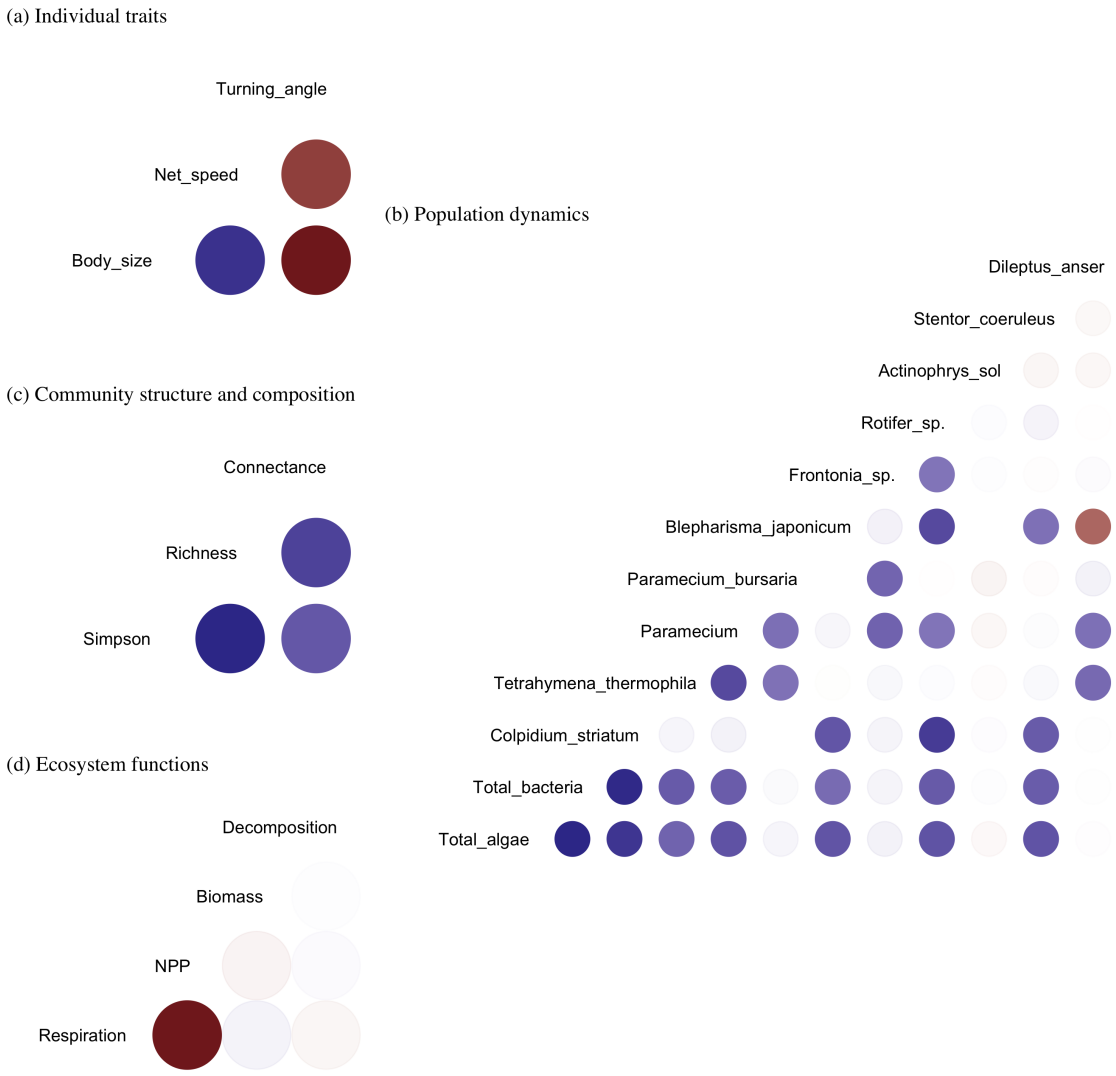


*Figure 3: Similarity in the responses to disturbances within levels of organisation: (a) individual, (b) population, (c) community and (d) ecosystem. Colours reveals correlation > 50%. The red describes a dissimilarity in the response while the blue describes a similarity in the response between variables.*

At the population level, a similarity/dissimilarity pattern between each population was more complex (Figure 3b). Nevertheless, I highlighted one bottom-up cluster

"algae-bacteria-*Colpidium*-*Tetrahymena*-*Paramecium*-*Blepharisma*-*Rotifer*-*Stentor*" that increased with only the organic matter enrichment in isolation (Figure 1). And another cluster "*Tetrahymena*-*Paramecium*-*P.bursaria*" was due to an effect of the light availability disturbance (Figure 1). Twelve other correlations between populations were also observed, most of them were positive except only one negative correlation between *Blepharisma japonicum* and *Dileptus anser* (Figure 3b).

At the community level, the similarity of the response is observed between the richness, the connectance and the Simpson's diversity index (Figure 3c) due to their global convergence in the response of disturbances (Figure 2). Indeed, when negative main effects were observed, the 2-, 3- and 4-way interaction terms were positive, negative and positive respectively (Figure 1).

At the ecosystem level, a dissimilarity was observed between the respiration and net primary production, due to opposing effect of increased organic matter enrichment (Figure 1). However, this observed dissimilarity was due to the negative correlation between NPP and respiration (i.e., a negative NPP corresponds to an increase in the community respiration).

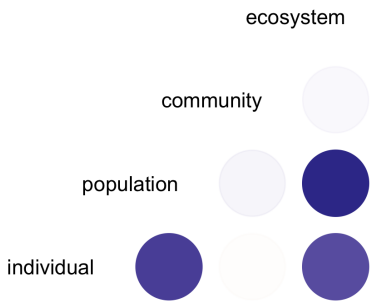


*Figure 4: Similarity in the response to disturbance between level of organisation. Colours reveals correlation > 50%. The red describes a dissimilarity in the response while the blue describes a similarity in the response between variables.*

Between the levels of organisation (Figure 4), I observed similar response pattern between the individual traits, population dynamics and ecosystem functions. This correlation was driven by the largest positive effect of organic matter enrichment (Figure 2).

# Discussion

Multiple environmental disturbances challenge ecological systems as they affect in isolation different components and might interact between each other. The main results of my experiment highlighted that 1) the four environmental disturbances affected differently all levels of organisation, 2) the interactions were relatively rare, 3) the presence of similarity of response to disturbances within population dynamics was evidence of bottom-up consequence on the food web and 4) the presence of similarity of response between levels of organisation could improve models to up-scale multiple environmental disturbances through levels of organisation.

In my experiment, the organic matter enrichment was the dominant disturbance that positively affected the microbial ecosystem. Indeed, this disturbance promoted the abundances of the resources (algae and bacteria), and therefore, the abundances of the consumers. However, the bottom-up effect was not observed on the predators' abundances (except for Stentor coeruleus, an omnivorous ciliate, which can also feed on resources and other ciliates (Laybourn 1976; Rapport *et al.* 1972)). Such bottom-up effect had been observed in natural stream food web where a large detrital input (i.e., high organic matter) enhanced population sizes at higher trophic level (Hall *et al.* 2000). By increasing the total abundance, the organic matter enrichment increased the biomass and the respiration of the ecosystem. Moreover, at the individual level, this disturbance increased the body size of Rotifer sp. and the net speed of *Colipidium striatum*, whereas it decreased the change of direction (i.e., turning angle) of the *Rotifer*

sp.. Note that *Rotifer* sp. had smaller speed and high turning angle (see Supplement), therefore a decrease in turning angle might be due to higher competition for food, as there was a higher total abundance of ciliates. However, this result contradicts the theory suggesting that animal should alter their behaviour to increase their energy intake. For example, the rotifer showed higher turning frequency in a resource-rich and a competitive environment (Kuefler *et al.* 2013).

The second largest disturbance on the population dynamics was the reduction in light availability (Figure 1). This disturbance reduced the algal abundances and consequently reduced the densities of the algivorous *Paramecium bursaria*. Note that this disturbance slightly reduced the abundance of the bacterivorous *Tetrahymena thermophila*, which can highlight an indirect effect of competition or predation. At the individual level, reduction in light availability affected only *Colpidium striatum* by increasing its body size and decreasing variation in direction (Figure 3). Surprisingly, reduction in light availability did not affect any community structure and composition, highlighting that the community remained stable. Indeed, this disturbance affected primarily the autotroph organisms, whereas my community was principally heterotroph. Therefore, I could have expected that the community became even more heterotroph, but autotroph consumers (with lower competition than heterotroph consumers) were affected although persisted.

The two other disturbances (i.e., increasing temperature and decreasing N:P ratio) did not impact the different levels of organisation greatly. I was expecting a greater effect of temperature as it plays an important role in physiological rates (metabolic and growth rates) and biological rates (interaction strength for competition and predation) which affect the whole community and therefore the ecosystem functions (Woodward *et al.* 2010). This weak effect might be due to the duration of the experiment and/or magnitude of change. Additionally, the identification of the species was based on control conditions. Then, if the traits changed with disturbances (especially organic matter

and temperature - unpublished data), there was a possibility of misidentification and might explain the few effects detected at the individual level of organisation. Therefore, some additional manual checking at different days and treatment should be considered to validate the method/results. Already, Pennekamp and collaborators (2017) highlighted that the abiotic context (i.e., temperature) decreased the classification success, and provided methods for adapting the classification process.

Despite the number of interactions tested with my full-factorial design, the interactions were rarely detected (8% for the individual traits, 8% for the population dynamics, 21% for the community structure and composition, and 0% for the ecosystem functions). The presence of interactions indicated a non-additive cumulative effect of multiple environmental disturbances. Therefore, in my experiment, non-additive cumulative effects could be found for the individual, population and community levels of organisation; whereas additive cumulative effect (i.e., absence of interactions) were found at the ecosystem level. This observation contradicted the results of the meta-analysis of paired stressors in freshwater ecosystems (Jackson *et al.* 2016). Indeed, they found prevalent evidence of additive and antagonistic cumulative effect for diversity (i.e., community metric) and functional metric respectively.

Among the interactions observed, the signs of interactions were opposite as the complexity of interactions increased (e.g., positive 2-way, negative 3-way and positive 4-way interactions for richness; Figure 1). Such opposite signs of interactions might question the process of statistical detection and/or the nature of the relationship between the effect and the number of disturbances. First, the process of statistical detection is incremental (with the ANOVA type III). Indeed, to detect a 3-way interaction depended on the detection of main effects and the 2-way interactions involved in the combination of three disturbances. Additionally, the detection of interaction also depends on the statistical power. Indeed, to detect higher a level of interaction, it is often recommended to increase the number of replicates to increase the statistical power (Leon & Heo 2009;

Heo & Leon 2010). Therefore, I could question whether the detection of 3- and 4-way interactions were an artefact of the statistics due to a large 2-way interaction term. Second, the nature of the relationship between the response (e.g., effect size) and the number of disturbances could lead to overestimating higher levels of interaction. Indeed, with additive cumulative effect, a linear relationship is expected. Whereas, with non-additive cumulative effect, the relationship can be nonlinear (Brennan & Collins 2015). For example, if the response is saturating (e.g., a system goes extinct with two disturbances), any additional disturbances could not change the response - except if antagonistic interactions are present - but 3- and 4-way interactions could be detected to counteract the strong 2-way interaction that led to the system extinction. Note that such reverse signs are difficult to observe in the literature, as very few studies look at more than two disturbances. Thus, more studies would be necessary to determine such pattern.

The presence of interactions between environmental drivers can be one source of unpredictability, along with feedback between the community and the environment (see Chapter 4). In most models used for ecosystem management (e.g., Allan *et al.* 2013), the additive hypothesis (i.e., absence of interactions) has the advantage of using the extended acquired knowledge of environmental drivers' effects in isolation. Furthermore, taking into account the type of interactions can lead to different scenarios (Sala *et al.* 2000). However, in my experiment, I highlighted that interactions were not widespread but nonetheless complex. Indeed, both synergistic and antagonistic interactions were observed. Therefore, it appeared that selecting one scenario of interacting environmental drivers may not be the optimal solution. Rather, future research should focus on the relationship between the ecosystem function (i.e., response variable of interest) and the number of environmental drivers. Indeed, the nature of the relationship could inform about the prevalence of interactions that depends on the levels/magnitudes of the disturbances.

The presence of similarity and dissimilarity of response to disturbances within levels of organisation could inform about the predictability of multiple environmental disturbances. Indeed, in the absence of overall effect (i.e., no correlation) or high variability (i.e., negative correlation), the system would not be predictable. Whereas, with positive correlation, when the components within a level of organisation respond similarly to disturbance, the system would be more predictable due to lower variability. However, the use of effect size Hedges'd (or any metric summarising effects between different variables) is driven by large effects that might underestimate the complexity of the system's responses and therefore overestimate its predictability.

Community structure is widely used to detect and monitor disturbances' effects on ecosystems (Warwick 1993; Attrill & Depledge 1997). In my experiment, the communities were relatively stable - when compared to the control treatment (e.g., extinctions were also observed in the control microcosms). Thus, qualitative information (i.e., presence/absence of particular species or link between species that describe richness and connectance respectively) were not as informative as quantitative information (e.g., abundances). Therefore, other components of the community taking into account quantitative information should be considerate to scale the response of disturbance across biological levels of organisation. For example, the functional diversity (Petchey & Gaston 2002; that can be based on trophic level or feeding strategies in my experiment) could be used to explore the link between community and ecosystem levels. Note that the similarity of response to disturbance is one criterion to build functional groups (Lavorel *et al.* 1997). The functional diversity already bridged the community and ecosystem levels in the diversity-stability debate. Indeed, the ecosystem function increased with diversity, but a plateau is reached due to functional redundancy (Yachi & Loreau 1999; Wohl *et al.* 2004; Kang *et al.* 2015).

To summarise, scaling the response to disturbances across biological levels of organisation, I used a correlation approach that highlighted two specific points: 1/ the

absence of correlation between community and individual/population/ecosystem (discussed above), and 2/ the large influence of one disturbance (i.e., organic matter enrichment) on the correlation between individual traits, populations dynamics and ecosystem functions, with a positive large mean effect size for these three biological levels of organisation. Note that when this disturbance was absent, the correlation was less strong (see Supplement). However, correlation does not imply causation. Therefore, more statistical and/or mathematical modelling should be considerate to inform how much effect size at a smaller level of organisation (e.g., body size) is transferred at a higher level of organisation (e.g., ecosystem functioning). One approach can be based on a response-effect framework (Suding *et al.* 2008). For example, a recent study showed that the community dynamic was better predicted with individual traits in the model (Griffiths *et al.* 2017). Another statistical approach would be to use structural equation modelling that can take into account direct and indirect effects of environmental change across components of levels of organisation (Antiqueira *et al.* 2018). Therefore, future research should consider integrating a response-effect framework or a structural equation model to global model (e.g., Earth System Model) to capture the complexity of the biological component (and its feedback on the environment) and tackle the challenge of predicting the effects of global change on ecological systems.

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# Supplement

## Algae identification

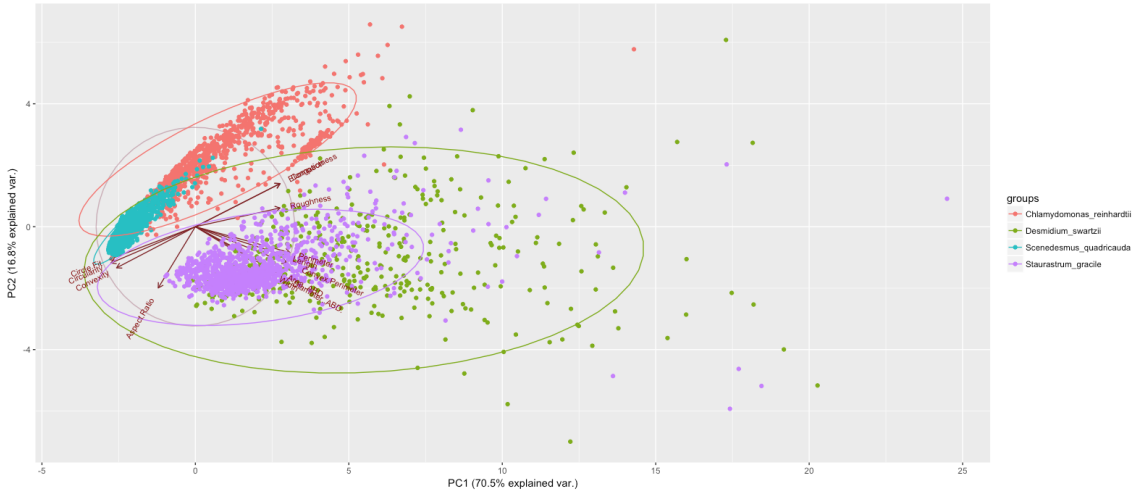



*Figure S1: Principal Component Analysis (PCA) on variables characterising the four algae species : area, aspect ratio, circle fit, circularity, compactiness, convex perimeter, diameter, elongation, length, roughness, perimeter and width.*

From the monocultures, I selected randomly 400 individuals to create a training dataset for the random forest using the variables above.

| | Chlamydomonas reinhardtii | Desmidium swartzii | Scenedesmus quadricauda | Staurastrum gracile | **classification error** |
|---|---|---|---|---|---|
| Chlamydomonas reinhardtii | 386 | 2 | 11 | 1 | **0.0350** |
| Desmidium swartzii | 2 | 332 | 0 | 66 | **0.1700** |
| Scenedesmus quadricauda | 13 | 0 | 387 | 0 | **0.0325** |
| Staurastrum gracile | 0 | 58 | 0 | 342 | **0.1450** |

The classification error among monocultures was variable, from 3 to 17%, which was correct (below 20%). And give an accuracy of 95.96%.

## Ciliates identification

I selected 7 videos at the beginning of the experiment (i.e., control condition), and identify manually ∼100 individuals (*Blepharisma*, *Colpidium*, *Euplotes*, *Frontonia*, *Paramecium*, *Rotifer* and *Tetrahymena*.)

### Random Forest - classification error

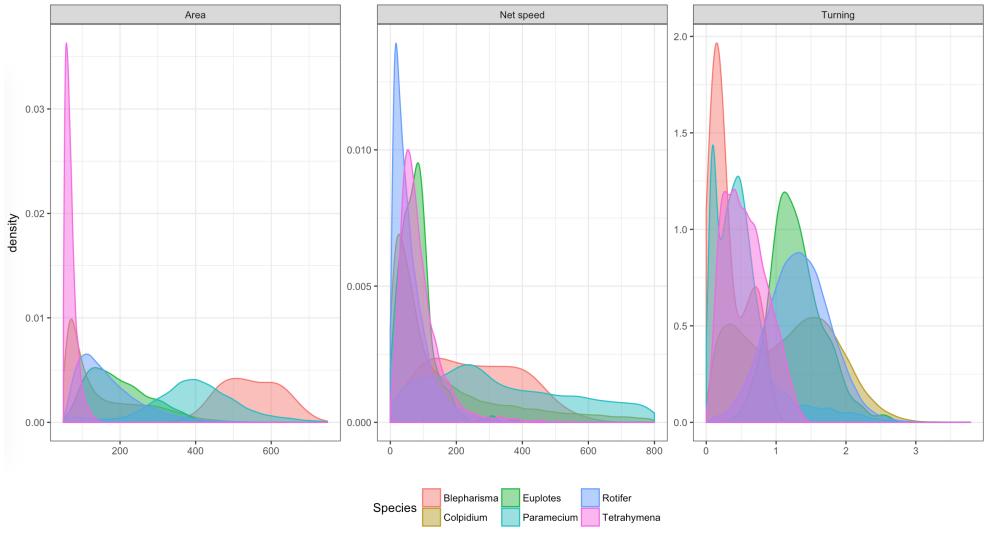
| | Blepharisma | Colpidium | Euplotes | Frontonia | Nassula | Paramecium | Rotifer | Tetrahymena | **classification error** |
|---|---|---|---|---|---|---|---|---|---|
| Blepharisma | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | **1.00** |
| Colpidium | 0 | 27 | 0 | 0 | 0 | 1 | 2 | 1 | **0.13** |
| Euplotes | 0 | 6 | 0 | 0 | 0 | 1 | 1 | 0 | **1.00** |
| Frontonia | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | **1.00** |
| Nassula | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | **1.00** |
| Paramecium | 2 | 2 | 0 | 0 | 0 | 41 | 1 | 0 | **0.07** |
| Rotifer | 0 | 1 | 0 | 0 | 0 | 1 | 12 | 0 | **0.14** |
| Tetrahymena | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 1 | **0.83** |

### Sector Vector Machine - classification error

| | Blepharisma | Colpidium | Euplotes | Frontonia | Nassula | Paramecium | Rotifer | Tetrahymena | **classification error** |
|---|---|---|---|---|---|---|---|---|---|
| Blepharisma | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **0.00** |
| Colpidium | 0 | 29 | 6 | 1 | 0 | 1 | 2 | 3 | **0.22** |
| Euplotes | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | **0.00** |
| Frontonia | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | **0.00** |
| Nassula | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **0.00** |
| Paramecium | 2 | 0 | 1 | 1 | 1 | 42 | 0 | 0 | **0.11** |
| Rotifer | 0 | 2 | 0 | 0 | 0 | 1 | 12 | 0 | **0.20** |
| Tetrahymena | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | **0.00** |

### Trait distribution

*Figure S2: Trait distribution of the different species identified.*



## Who feeds on whom?

| **Species** | *Rotifer* sp. | *Blepharisma japonicum* | *Euplotes* sp. | *Frontonia* sp. | *Stentor coeruleus* | *Actinophrys sol* | *Dileptus anser* | *Paramecium caudatum* | *Paramecium bursaria* | *Tetrahymena thermophila* | *Colpidium striatum* | *Euplotes daidaleos* | *Nassula aurea* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Serratia fonticola* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| *Bacillus subtilis* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| *Chlamydomonas reinhardtii* | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| *Scenedesmus quadricauda* | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| *Staurastrum gracile* | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| *Desmidium swartzii* | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| *Rotifer* sp. | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Blepharisma japonicum* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Euplotes* sp. | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Frontonia* sp. | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Stentor coeruleus* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Actinophrys sol* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Dileptus anser* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Paramecium caudatum* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Paramecium bursaria* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Tetrahymena thermophila* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Colpidium striatum* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Euplotes daidaleos* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Nassula aurea* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |

# Body size of *Colpidium striatum*

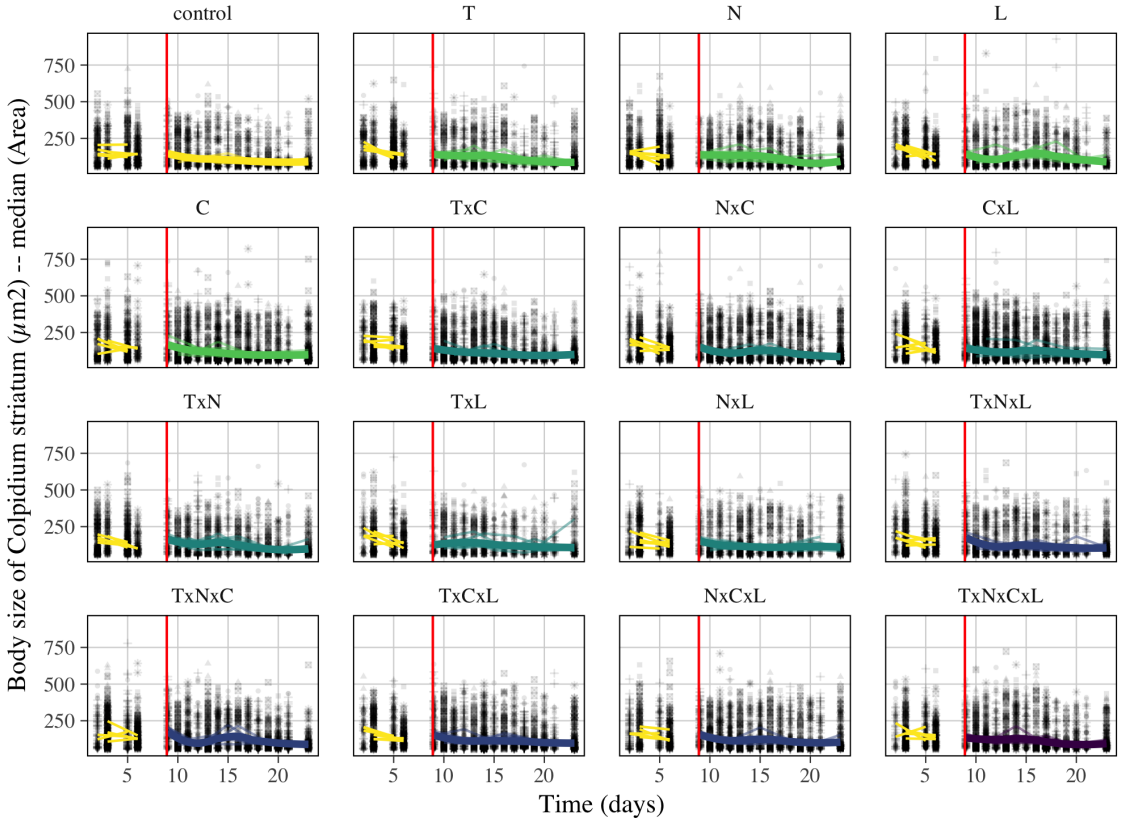



*Figure S3: Time serie of the body size ($\mu m^2$) of Colpidium striatum. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.$L^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.$L^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S1: Statisitcal analysis of the body size of Colpidium striatum. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 14% of deviance (Adj.* $R^2$ *= 6.5%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 137326.000 | 1 | 207.30 | <0.001 *** | -0.002 | 0.018 | -0.09 | 0.930 | s(day):treatmentno.change | 1.000 | 1.000 | 0.001 | 0.971 |
| Temperature (T) | 14.000 | 1 | 0.02 | 0.885 | 0.029 | 0.025 | 1.16 | 0.249 | s(day):treatmentT | 1.000 | 1.000 | 0.431 | 0.512 |
| Nutrient (N) | 1746.000 | 1 | 2.64 | 0.108 | 0.030 | 0.025 | 1.18 | 0.239 | s(day):treatmentN | 1.041 | 1.082 | 0.206 | 0.644 |
| Carbon (C) | 60.000 | 1 | 0.09 | 0.764 | 0.038 | 0.025 | 1.51 | 0.133 | s(day):treatmentC | 1.000 | 1.000 | 0.126 | 0.723 |
| Light (L) | 477.000 | 1 | 0.72 | 0.399 | 0.065 | 0.025 | 2.56 | 0.011 * | s(day):treatmentL | 2.994 | 3.698 | 3.149 | 0.019 * |
| T:N | 109.000 | 1 | 0.16 | 0.686 | -0.001 | 0.036 | -0.03 | 0.975 | s(day):treatmentTN | 1.000 | 1.000 | 0.447 | 0.504 |
| T:C | 1038.000 | 1 | 1.57 | 0.214 | -0.037 | 0.036 | -1.03 | 0.305 | s(day):treatmentTC | 1.000 | 1.000 | 0.052 | 0.820 |
| N:C | 990.000 | 1 | 1.49 | 0.225 | -0.037 | 0.036 | -1.04 | 0.297 | s(day):treatmentNC | 2.075 | 2.579 | 1.144 | 0.374 |
| T:L | 302.000 | 1 | 0.46 | 0.502 | -0.011 | 0.036 | -0.30 | 0.767 | s(day):treatmentTL | 1.000 | 1.000 | 3.634 | 0.057 |
| N:L | 1660.000 | 1 | 2.51 | 0.117 | -0.037 | 0.036 | -1.04 | 0.298 | s(day):treatmentNL | 1.000 | 1.000 | 4.895 | 0.027 * |
| C:L | 343.000 | 1 | 0.52 | 0.474 | -0.057 | 0.036 | -1.58 | 0.116 | s(day):treatmentCL | 1.000 | 1.000 | 0.585 | 0.445 |
| T:N:C | 887.000 | 1 | 1.34 | 0.251 | 0.015 | 0.051 | 0.29 | 0.771 | s(day):treatmentTNC | 5.405 | 6.494 | 2.688 | 0.012 * |
| T:N:L | 324.000 | 1 | 0.49 | 0.486 | -0.022 | 0.051 | -0.43 | 0.667 | s(day):treatmentTNL | 1.000 | 1.000 | 0.895 | 0.345 |
| T:C:L | 1401.000 | 1 | 2.12 | 0.150 | 0.021 | 0.051 | 0.41 | 0.684 | s(day):treatmentTCL | 1.000 | 1.000 | 0.818 | 0.366 |
| N:C:L | 873.000 | 1 | 1.32 | 0.255 | 0.042 | 0.051 | 0.82 | 0.414 | s(day):treatmentNCL | 1.000 | 1.000 | 1.333 | 0.249 |
| T:N:C:L | 861.000 | 1 | 1.30 | 0.258 | -0.011 | 0.072 | -0.16 | 0.875 | s(day):treatmentTNCL | 1.000 | 1.000 | 0.182 | 0.670 |
| Residuals | 52997.000 | 80 | | | | | | | | | | | |

## Body size of *Rotifer* sp.

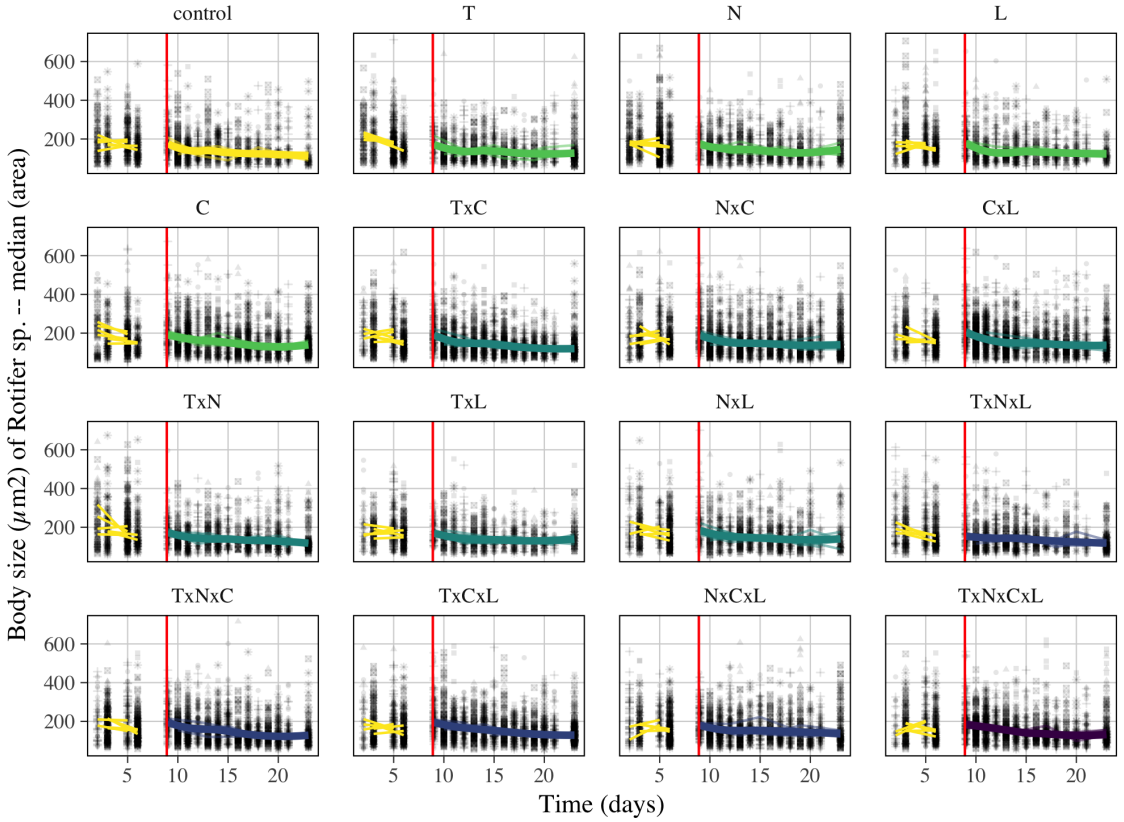



*Figure S4: Time serie of the body size ($\mu m^2$) of Rotifer sp. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.$L^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.$L^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S2: Statisitcal analysis of the body size of Rotifer sp. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e., **Pre-perturbation**). The time series were analysed with a GAM explaining 25.7% of deviance (Adj. $R^2$ = 18.3%), with the main effects and their interactions as **parametric coefficients** and the temporal change with a spline function the for each treatment (i.e., **smooth term**).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 180953 | 1 | 484.56 | <0.001 *** | -0.002 | 0.010 | -0.21 | 0.835 | s(day):treatmentno.change | 1.000 | 1.000 | 0.002 | 0.963 |
| Temperature (T) | 1 | 1 | 0.00 | 0.964 | 0.016 | 0.014 | 1.15 | 0.250 | s(day):treatmentT | 2.827 | 3.496 | 3.019 | 0.019 * |
| Nutrient (N) | 94 | 1 | 0.25 | 0.617 | 0.038 | 0.014 | 2.73 | 0.007 ** | s(day):treatmentN | 2.877 | 3.556 | 2.275 | 0.058 |
| Carbon (C) | 21 | 1 | 0.06 | 0.813 | 0.063 | 0.014 | 4.46 | <0.001 *** | s(day):treatmentC | 3.315 | 4.084 | 2.003 | 0.091 |
| Light (L) | 283 | 1 | 0.76 | 0.387 | 0.012 | 0.014 | 0.85 | 0.394 | s(day):treatmentL | 1.000 | 1.000 | 2.267 | 0.133 |
| T:N | 98 | 1 | 0.26 | 0.610 | -0.030 | 0.020 | -1.52 | 0.128 | s(day):treatmentTN | 1.000 | 1.000 | 0.351 | 0.554 |
| T:C | 42 | 1 | 0.11 | 0.739 | -0.054 | 0.020 | -2.71 | 0.007 ** | s(day):treatmentTC | 1.000 | 1.000 | 0.719 | 0.397 |
| N:C | 30 | 1 | 0.08 | 0.779 | -0.039 | 0.020 | -1.98 | 0.049 * | s(day):treatmentNC | 1.112 | 1.215 | 2.129 | 0.116 |
| T:L | 260 | 1 | 0.70 | 0.406 | 0.001 | 0.020 | 0.05 | 0.958 | s(day):treatmentTL | 2.065 | 2.569 | 3.549 | 0.029 * |
| N:L | 527 | 1 | 1.41 | 0.239 | -0.007 | 0.020 | -0.34 | 0.735 | s(day):treatmentNL | 1.000 | 1.000 | 0.457 | 0.499 |
| C:L | 18 | 1 | 0.05 | 0.829 | -0.011 | 0.020 | -0.54 | 0.589 | s(day):treatmentCL | 1.431 | 1.741 | 0.226 | 0.744 |
| T:N:C | 206 | 1 | 0.55 | 0.460 | 0.052 | 0.028 | 1.85 | 0.065 | s(day):treatmentTNC | 2.765 | 3.421 | 1.151 | 0.269 |
| T:N:L | 818 | 1 | 2.19 | 0.143 | -0.020 | 0.028 | -0.71 | 0.479 | s(day):treatmentTNL | 1.000 | 1.000 | 1.531 | 0.217 |
| T:C:L | 218 | 1 | 0.58 | 0.447 | 0.040 | 0.028 | 1.41 | 0.160 | s(day):treatmentTCL | 1.000 | 1.001 | 0.407 | 0.524 |
| N:C:L | 73 | 1 | 0.20 | 0.659 | 0.006 | 0.028 | 0.22 | 0.824 | s(day):treatmentNCL | 1.000 | 1.000 | 3.046 | 0.082 |
| T:N:C:L | 477 | 1 | 1.28 | 0.262 | -0.018 | 0.040 | -0.46 | 0.647 | s(day):treatmentTNCL | 3.530 | 4.341 | 2.198 | 0.074 |
| Residuals | 29875 | 80 | | | | | | | | | | | |

## Turning angle of *Colpidium striatum*

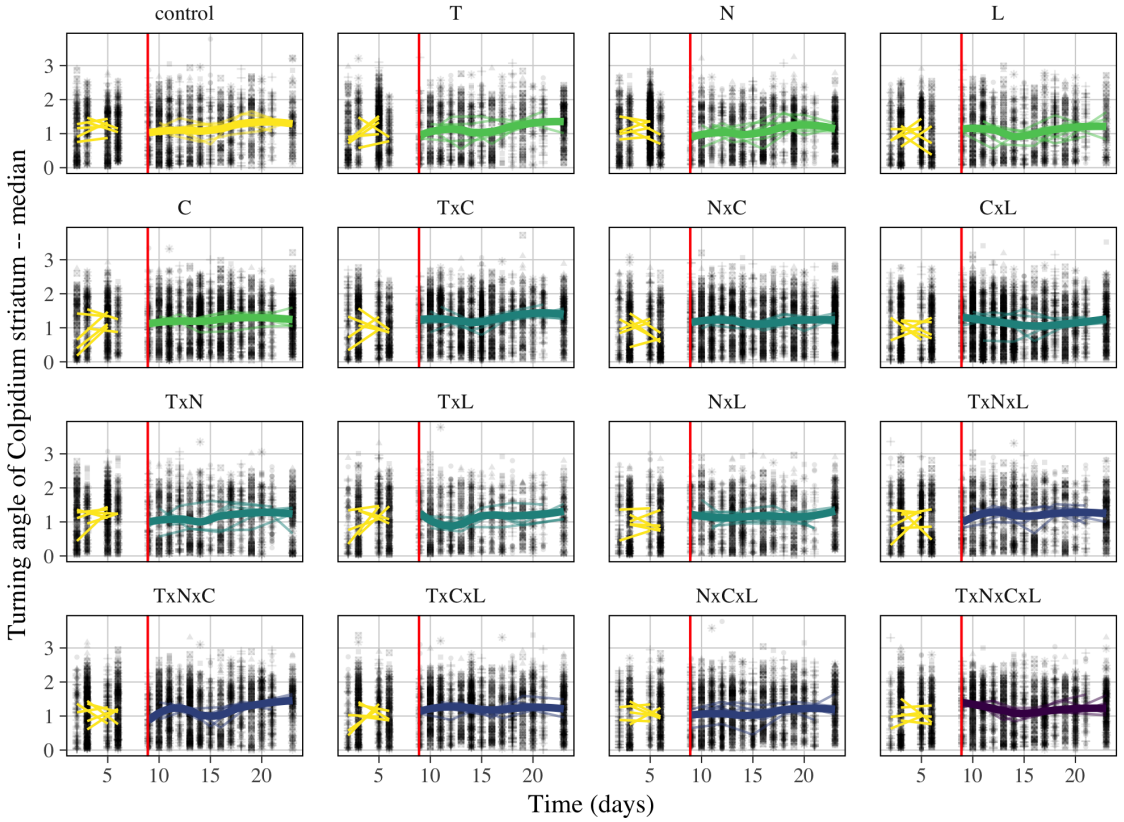



*Figure S5: Time serie of the turning angle of Colpidium striatum. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S3: Statisitcal analysis of the turning angle of Colpidium striatum. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 20.6% of deviance (Adj. $R^2$ = 12.4%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 8.225 | 1 | 180.31 | <0.001 *** | 0.000 | 0.008 | 0.00 | 0.998 | s(day):treatmentno.change | 1.000 | 1.000 | 0.033 | 0.855 |
| Temperature (T) | 0.000 | 1 | 0.00 | 0.973 | -0.004 | 0.011 | -0.37 | 0.715 | s(day):treatmentT | 1.000 | 1.000 | 0.383 | 0.537 |
| Nutrient (N) | 0.001 | 1 | 0.02 | 0.898 | -0.014 | 0.011 | -1.27 | 0.204 | s(day):treatmentN | 1.000 | 1.000 | 0.002 | 0.966 |
| Carbon (C) | 0.010 | 1 | 0.22 | 0.638 | 0.005 | 0.011 | 0.46 | 0.643 | s(day):treatmentC | 1.000 | 1.000 | 2.320 | 0.128 |
| Light (L) | 0.039 | 1 | 0.86 | 0.357 | -0.025 | 0.011 | -2.20 | 0.029 * | s(day):treatmentL | 2.161 | 2.684 | 2.341 | 0.101 |
| T:N | 0.014 | 1 | 0.31 | 0.581 | 0.006 | 0.016 | 0.35 | 0.726 | s(day):treatmentTN | 1.000 | 1.000 | 0.044 | 0.834 |
| T:C | 0.003 | 1 | 0.06 | 0.812 | 0.015 | 0.016 | 0.97 | 0.335 | s(day):treatmentTC | 1.192 | 1.357 | 0.133 | 0.848 |
| N:C | 0.037 | 1 | 0.81 | 0.372 | 0.012 | 0.016 | 0.73 | 0.467 | s(day):treatmentNC | 1.010 | 1.020 | 3.242 | 0.073 |
| T:L | 0.026 | 1 | 0.56 | 0.455 | 0.015 | 0.016 | 0.93 | 0.352 | s(day):treatmentTL | 4.531 | 5.515 | 1.752 | 0.134 |
| N:L | 0.002 | 1 | 0.03 | 0.856 | 0.032 | 0.016 | 1.98 | 0.049 * | s(day):treatmentNL | 4.213 | 5.147 | 3.153 | 0.008 ** |
| C:L | 0.001 | 1 | 0.02 | 0.879 | 0.013 | 0.016 | 0.80 | 0.423 | s(day):treatmentCL | 2.068 | 2.571 | 3.130 | 0.049 * |
| T:N:C | 0.024 | 1 | 0.52 | 0.472 | -0.015 | 0.023 | -0.68 | 0.494 | s(day):treatmentTNC | 4.640 | 5.640 | 2.282 | 0.043 * |
| T:N:L | 0.011 | 1 | 0.23 | 0.633 | -0.004 | 0.023 | -0.17 | 0.869 | s(day):treatmentTNL | 1.001 | 1.001 | 1.955 | 0.163 |
| T:C:L | 0.000 | 1 | 0.00 | 0.976 | -0.014 | 0.023 | -0.63 | 0.528 | s(day):treatmentTCL | 1.000 | 1.000 | 3.268 | 0.071 |
| N:C:L | 0.062 | 1 | 1.36 | 0.247 | -0.033 | 0.023 | -1.44 | 0.150 | s(day):treatmentNCL | 1.000 | 1.000 | 0.691 | 0.406 |
| T:N:C:L | 0.029 | 1 | 0.63 | 0.430 | 0.020 | 0.032 | 0.63 | 0.531 | s(day):treatmentTNCL | 1.786 | 2.222 | 3.470 | 0.029 * |
| Residuals | 3.649 | 80 | | | | | | | | | | | |

## Turning angle of *Tetrahymena thermophila*

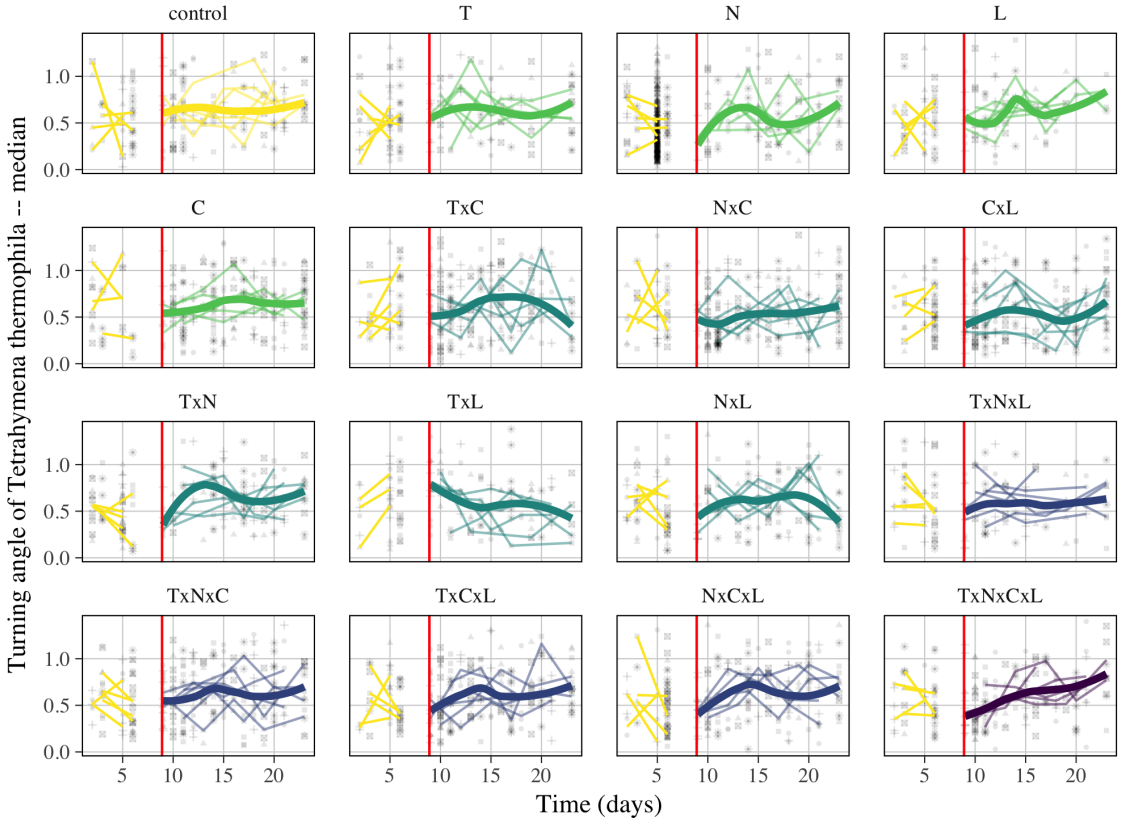



*Figure S6: Time serie of the turning angle of Tetrahymena thermophila. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S4: Statisitcal analysis of the turning angle of Tetrahymena thermophila. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e., **Pre-perturbation**). The time series were analysed with a GAM explaining 20.3% of deviance (Adj. $R^2$ = 11.7%), with the main effects and their interactions as **parametric coefficients** and the temporal change with a spline function the for each treatment (i.e., **smooth term**).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 1.659 | 1 | 64.88 | < 0.001 *** | 0.000 | 0.010 | 0.00 | 0.997 | s(day):treatmentno.change | 1.000 | 1.000 | 0.000 | 0.992 |
| Temperature (T) | 0.000 | 1 | 0.00 | 1.000 | -0.004 | 0.015 | -0.26 | 0.794 | s(day):treatmentT | 1.000 | 1.000 | 0.861 | 0.354 |
| Nutrient (N) | 0.000 | 1 | 0.00 | 0.971 | -0.028 | 0.015 | -1.93 | 0.055 | s(day):treatmentN | 3.522 | 4.350 | 1.742 | 0.134 |
| Carbon (C) | 0.000 | 1 | 0.01 | 0.921 | -0.008 | 0.014 | -0.56 | 0.579 | s(day):treatmentC | 1.434 | 1.746 | 0.215 | 0.745 |
| Light (L) | 0.000 | 1 | 0.00 | 0.986 | 0.000 | 0.015 | -0.01 | 0.996 | s(day):treatmentL | 1.000 | 1.000 | 1.035 | 0.310 |
| T:N | 0.000 | 1 | 0.00 | 0.949 | 0.023 | 0.021 | 1.12 | 0.264 | s(day):treatmentTN | 3.368 | 4.153 | 1.840 | 0.119 |
| T:C | 0.019 | 1 | 0.75 | 0.388 | -0.004 | 0.020 | -0.19 | 0.853 | s(day):treatmentTC | 1.359 | 1.634 | 0.166 | 0.712 |
| N:C | 0.016 | 1 | 0.64 | 0.427 | -0.004 | 0.020 | -0.19 | 0.852 | s(day):treatmentNC | 1.000 | 1.000 | 0.223 | 0.637 |
| T:L | 0.027 | 1 | 1.07 | 0.304 | -0.038 | 0.021 | -1.79 | 0.075 | s(day):treatmentTL | 1.000 | 1.000 | 16.955 | <0.001 *** |
| N:L | 0.003 | 1 | 0.11 | 0.741 | 0.018 | 0.021 | 0.84 | 0.403 | s(day):treatmentNL | 2.201 | 2.727 | 1.871 | 0.167 |
| C:L | 0.007 | 1 | 0.28 | 0.598 | -0.031 | 0.021 | -1.53 | 0.127 | s(day):treatmentCL | 3.625 | 4.445 | 2.180 | 0.067 |
| T:N:C | 0.030 | 1 | 1.15 | 0.286 | 0.009 | 0.028 | 0.33 | 0.740 | s(day):treatmentTNC | 1.000 | 1.000 | 0.335 | 0.563 |
| T:N:L | 0.021 | 1 | 0.83 | 0.365 | 0.017 | 0.030 | 0.57 | 0.569 | s(day):treatmentTNL | 1.000 | 1.000 | 0.524 | 0.470 |
| T:C:L | 0.099 | 1 | 3.85 | 0.053 | 0.069 | 0.029 | 2.40 | 0.017 * | s(day):treatmentTCL | 1.000 | 1.001 | 0.512 | 0.475 |
| N:C:L | 0.053 | 1 | 2.07 | 0.154 | 0.048 | 0.029 | 1.68 | 0.094 | s(day):treatmentNCL | 2.718 | 3.363 | 1.421 | 0.229 |
| T:N:C:L | 0.118 | 1 | 4.61 | 0.035 * | -0.077 | 0.041 | -1.88 | 0.061 | s(day):treatmentTNCL | 1.563 | 1.923 | 3.040 | 0.072 |
| Residuals | 2.046 | 80 | | | | | | | | | | | |

## Turning angle of *Rotifer* sp.

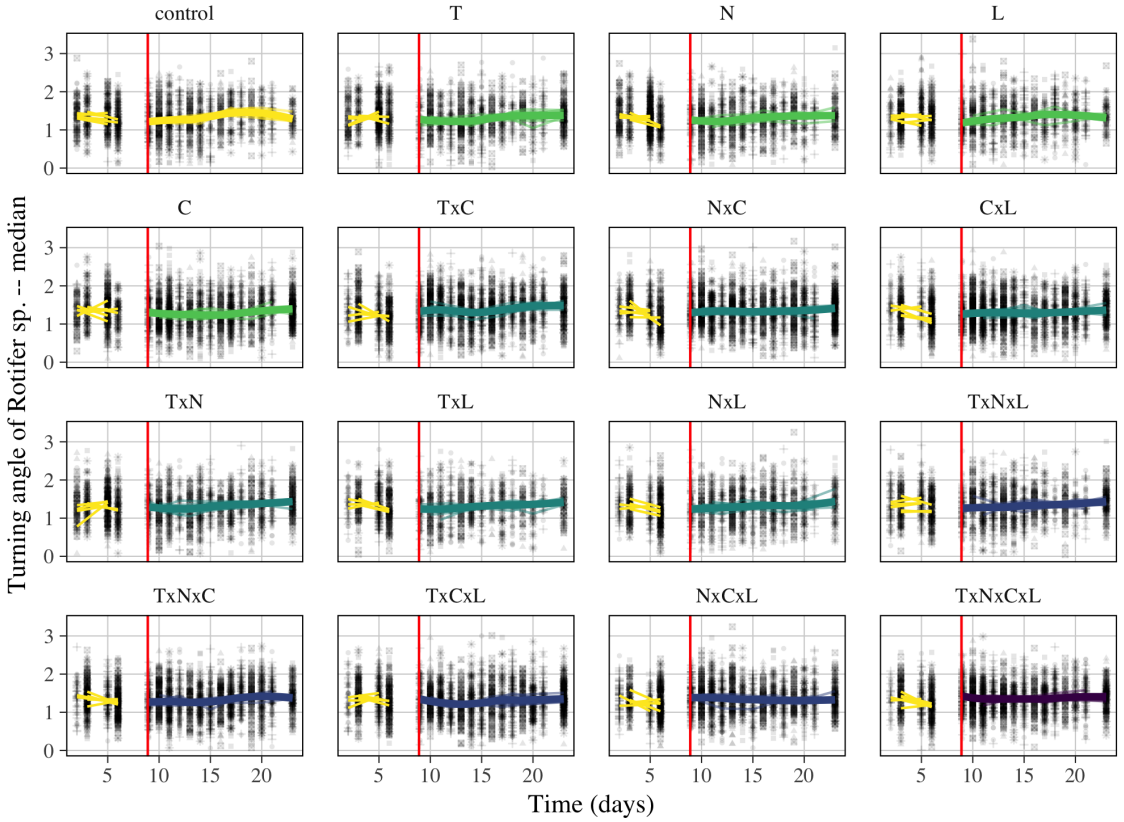



*Figure S7: Time serie of the turning angle of Rotifer sp. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S5: Statisitcal analysis of the turning angle of Rotifer sp. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 43.5% of deviance (Adj.* $R^2$ *= 35.1%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | | edf | Ref.df | F | p-value |
| (Intercept) | 10.140 | 1 | 1316.54 | <0.001 *** | 0.000 | 0.004 | 0.05 | 0.959 | | s(day):treatmentno.change | 1.000 | 1.001 | 0.008 | 0.931 |
| Temperature (T) | 0.002 | 1 | 0.26 | 0.612 | -0.010 | 0.005 | -1.91 | 0.057 | . | s(day):treatmentT | 2.942 | 3.635 | 2.931 | 0.021 * |
| Nutrient (N) | 0.000 | 1 | 0.01 | 0.909 | -0.009 | 0.005 | -1.83 | 0.068 | | s(day):treatmentN | 2.745 | 3.396 | 1.750 | 0.119 |
| Carbon (C) | 0.002 | 1 | 0.20 | 0.658 | -0.011 | 0.005 | -2.22 | 0.027 * | | s(day):treatmentC | 3.331 | 4.103 | 5.449 | <0.001 *** |
| Light (L) | 0.000 | 1 | 0.00 | 0.987 | -0.006 | 0.005 | -1.19 | 0.234 | | s(day):treatmentL | 2.619 | 3.243 | 1.442 | 0.234 |
| T:N | 0.002 | 1 | 0.21 | 0.651 | 0.014 | 0.007 | 2.00 | 0.046 * | | s(day):treatmentTN | 2.696 | 3.336 | 3.194 | 0.018 * |
| T:C | 0.014 | 1 | 1.85 | 0.177 | 0.027 | 0.007 | 3.77 | <0.001 *** | | s(day):treatmentTC | 3.316 | 4.085 | 5.261 | <0.001 *** |
| N:C | 0.000 | 1 | 0.00 | 0.945 | 0.019 | 0.007 | 2.67 | 0.008 ** | | s(day):treatmentNC | 3.510 | 4.317 | 5.249 | <0.001 *** |
| T:L | 0.000 | 1 | 0.01 | 0.917 | 0.009 | 0.007 | 1.23 | 0.219 | | s(day):treatmentTL | 3.053 | 3.764 | 2.497 | 0.038 * |
| N:L | 0.001 | 1 | 0.07 | 0.799 | 0.010 | 0.007 | 1.35 | 0.179 | | s(day):treatmentNL | 3.942 | 4.829 | 6.254 | <0.001 *** |
| C:L | 0.000 | 1 | 0.00 | 0.991 | 0.010 | 0.007 | 1.39 | 0.166 | | s(day):treatmentCL | 3.382 | 4.164 | 4.502 | 0.001 ** |
| T:N:C | 0.004 | 1 | 0.56 | 0.457 | -0.034 | 0.010 | -3.34 | 0.001 *** | | s(day):treatmentTNC | 2.528 | 3.132 | 2.651 | 0.049 * |
| T:N:L | 0.000 | 1 | 0.00 | 0.954 | -0.011 | 0.010 | -1.06 | 0.289 | | s(day):treatmentTNL | 3.198 | 3.943 | 3.751 | 0.005 ** |
| T:C:L | 0.009 | 1 | 1.16 | 0.285 | -0.032 | 0.010 | -3.13 | 0.002 ** | | s(day):treatmentTCL | 2.954 | 3.650 | 5.417 | <0.001 *** |
| N:C:L | 0.002 | 1 | 0.30 | 0.583 | -0.014 | 0.010 | -1.39 | 0.165 | | s(day):treatmentNCL | 3.153 | 3.890 | 8.569 | <0.001 *** |
| T:N:C:L | 0.004 | 1 | 0.49 | 0.487 | 0.041 | 0.014 | 2.87 | 0.004 ** | | s(day):treatmentTNCL | 2.915 | 3.602 | 5.597 | <0.001 *** |
| Residuals | 0.616 | 80 | | | | | | | | | | | | |

# Net speed of *Colpidium striatum*

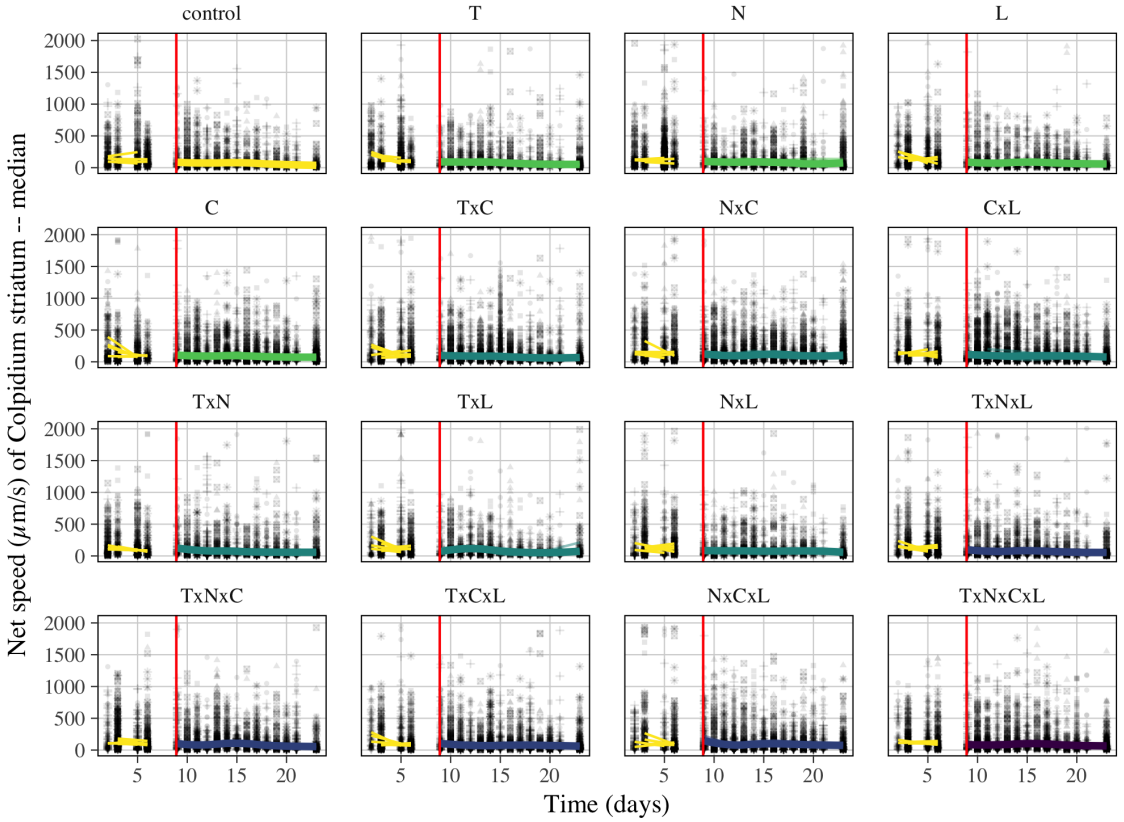



*Figure S8: Time serie of the net speed ($\mu m.sec^{-1}$) of Colpidium striatum. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.$L^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.$L^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S6: Statisitcal analysis of the net speed of Colpidium striatum. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 34.4% of deviance (Adj. $R^2$ = 27.2%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 96263 | 1 | 109.53 | <0.001 *** | -0.004 | 0.026 | -0.16 | 0.870 | s(day):treatmentno.change | 1.000 | 1.000 | 0.007 | 0.932 |
| Temperature (T) | 976 | 1 | 1.11 | 0.295 | 0.029 | 0.037 | 0.78 | 0.434 | s(day):treatmentT | 2.001 | 2.488 | 1.267 | 0.355 |
| Nutrient (N) | 291 | 1 | 0.33 | 0.567 | 0.064 | 0.037 | 1.75 | 0.081 | s(day):treatmentN | 2.158 | 2.681 | 2.541 | 0.081 |
| Carbon (C) | 3253 | 1 | 3.70 | 0.058 | 0.154 | 0.037 | 4.20 | <0.001 *** | s(day):treatmentC | 1.515 | 1.862 | 2.391 | 0.066 |
| Light (L) | 449 | 1 | 0.51 | 0.477 | 0.072 | 0.037 | 1.95 | 0.052 | s(day):treatmentL | 1.000 | 1.000 | 5.257 | 0.022 * |
| T:N | 0 | 1 | 0.00 | 0.988 | -0.038 | 0.052 | -0.73 | 0.466 | s(day):treatmentTN | 2.324 | 2.883 | 2.166 | 0.080 |
| T:C | 2877 | 1 | 3.27 | 0.074 | -0.087 | 0.052 | -1.68 | 0.094 | s(day):treatmentTC | 1.000 | 1.001 | 0.624 | 0.430 |
| N:C | 3098 | 1 | 3.53 | 0.064 | -0.011 | 0.052 | -0.22 | 0.826 | s(day):treatmentNC | 1.726 | 2.146 | 5.711 | 0.003 ** |
| T:L | 383 | 1 | 0.44 | 0.511 | -0.056 | 0.052 | -1.08 | 0.279 | s(day):treatmentTL | 5.019 | 6.069 | 6.150 | <0.001 *** |
| N:L | 426 | 1 | 0.48 | 0.489 | -0.065 | 0.052 | -1.24 | 0.215 | s(day):treatmentNL | 3.697 | 4.539 | 2.837 | 0.022 * |
| C:L | 1676 | 1 | 1.91 | 0.171 | -0.065 | 0.052 | -1.26 | 0.209 | s(day):treatmentCL | 2.401 | 2.977 | 2.958 | 0.028 * |
| T:N:C | 1163 | 1 | 1.32 | 0.254 | -0.026 | 0.073 | -0.36 | 0.719 | s(day):treatmentTNC | 1.000 | 1.000 | 1.086 | 0.298 |
| T:N:L | 46 | 1 | 0.05 | 0.820 | 0.027 | 0.073 | 0.37 | 0.709 | s(day):treatmentTNL | 2.121 | 2.635 | 2.980 | 0.041 * |
| T:C:L | 2171 | 1 | 2.47 | 0.120 | 0.049 | 0.073 | 0.67 | 0.506 | s(day):treatmentTCL | 1.806 | 2.248 | 2.572 | 0.060 |
| N:C:L | 2661 | 1 | 3.03 | 0.086 | 0.013 | 0.073 | 0.18 | 0.858 | s(day):treatmentNCL | 2.940 | 3.632 | 4.969 | 0.002 ** |
| T:N:C:L | 1550 | 1 | 1.76 | 0.188 | 0.057 | 0.104 | 0.55 | 0.581 | s(day):treatmentTNCL | 1.000 | 1.000 | 5.092 | 0.025 * |
| Residuals | 70309 | 80 | | | | | | | | | | | |

# Net speed of *Paramecium*

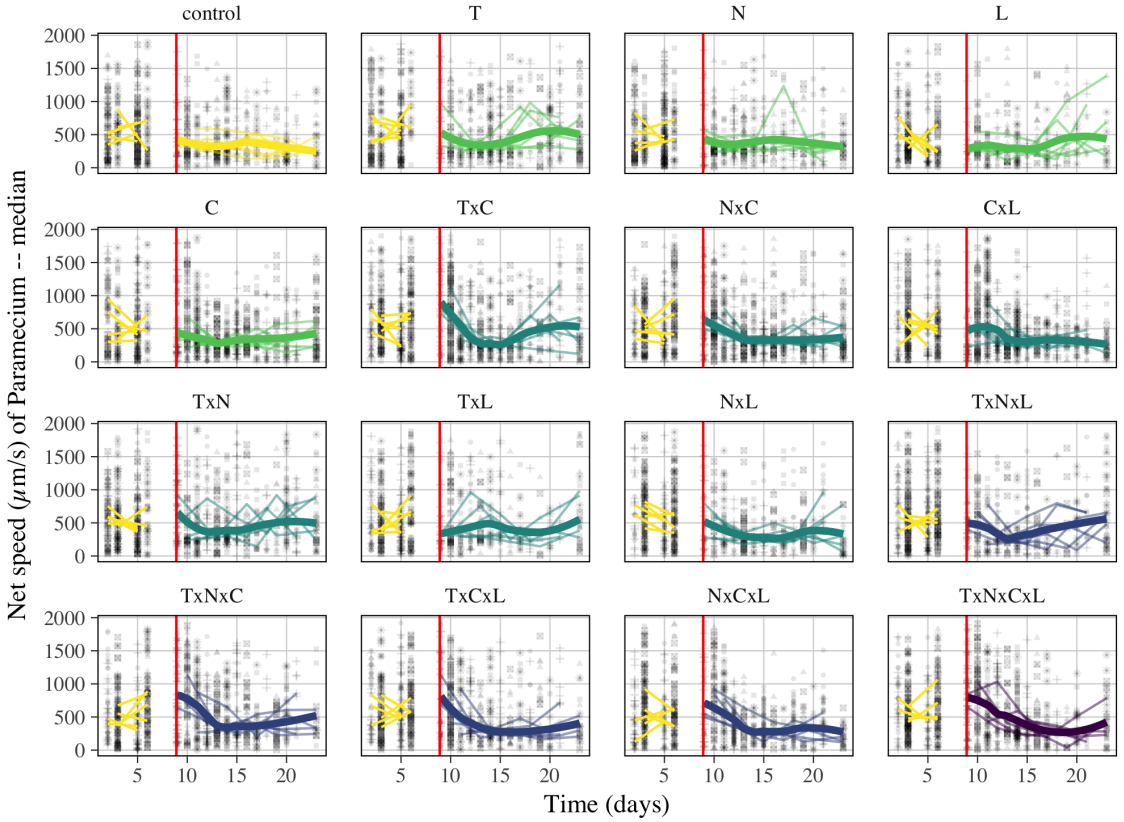



*Figure S9: Time serie of the net speed ($\mu m.sec^{-1}$) of Paramecium. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.$L^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.$L^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S7: Statisitcal analysis of the net speed of Paramecium. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e., **Pre-perturbation**). The time series were analysed with a GAM explaining 21% of deviance (Adj. $R^2$ = 13.8%), with the main effects and their interactions as **parametric coefficients** and the temporal change with a spline function the for each treatment (i.e., **smooth term**).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 1570699 | 1 | 105.18 | <0.001 *** | 0.001 | 0.037 | 0.03 | 0.980 | s(day):treatmentno.change | 1.000 | 1.000 | 0.000 | 0.985 |
| Temperature (T) | 660 | 1 | 0.04 | 0.834 | 0.146 | 0.052 | 2.79 | 0.005 ** | s(day):treatmentT | 1.000 | 1.000 | 6.993 | 0.008 ** |
| Nutrient (N) | 12647 | 1 | 0.85 | 0.360 | 0.035 | 0.052 | 0.68 | 0.497 | s(day):treatmentN | 1.000 | 1.000 | 0.294 | 0.588 |
| Carbon (C) | 761 | 1 | 0.05 | 0.822 | 0.018 | 0.052 | 0.35 | 0.726 | s(day):treatmentC | 1.000 | 1.000 | 1.170 | 0.280 |
| Light (L) | 44155 | 1 | 2.96 | 0.089 | 0.007 | 0.052 | 0.14 | 0.891 | s(day):treatmentL | 1.352 | 1.621 | 5.513 | 0.005 ** |
| T:N | 1437 | 1 | 0.10 | 0.757 | -0.031 | 0.074 | -0.42 | 0.673 | s(day):treatmentTN | 1.000 | 1.000 | 5.569 | 0.019 * |
| T:C | 2573 | 1 | 0.17 | 0.679 | -0.053 | 0.074 | -0.73 | 0.469 | s(day):treatmentTC | 3.717 | 4.564 | 2.885 | 0.018 * |
| N:C | 8372 | 1 | 0.56 | 0.456 | 0.030 | 0.074 | 0.41 | 0.682 | s(day):treatmentNC | 1.249 | 1.456 | 0.187 | 0.820 |
| T:L | 12446 | 1 | 0.83 | 0.364 | -0.059 | 0.074 | -0.80 | 0.424 | s(day):treatmentTL | 1.000 | 1.000 | 2.991 | 0.084 |
| N:L | 83143 | 1 | 5.57 | 0.021 * | -0.038 | 0.074 | -0.52 | 0.606 | s(day):treatmentNL | 1.000 | 1.000 | 0.002 | 0.963 |
| C:L | 42026 | 1 | 2.81 | 0.097 | -0.004 | 0.074 | -0.06 | 0.956 | s(day):treatmentCL | 1.000 | 1.000 | 0.642 | 0.423 |
| T:N:C | 4193 | 1 | 0.28 | 0.598 | -0.004 | 0.104 | -0.04 | 0.970 | s(day):treatmentTNC | 2.086 | 2.592 | 0.744 | 0.377 |
| T:N:L | 23136 | 1 | 1.55 | 0.217 | -0.003 | 0.105 | -0.03 | 0.975 | s(day):treatmentTNL | 2.072 | 2.576 | 1.792 | 0.118 |
| T:C:L | 13139 | 1 | 0.88 | 0.351 | -0.014 | 0.105 | -0.14 | 0.893 | s(day):treatmentTCL | 2.245 | 2.786 | 1.511 | 0.158 |
| N:C:L | 45683 | 1 | 3.06 | 0.084 | -0.038 | 0.104 | -0.36 | 0.716 | s(day):treatmentNCL | 1.407 | 1.705 | 5.459 | 0.014 * |
| T:N:C:L | 20681 | 1 | 1.38 | 0.243 | 0.072 | 0.148 | 0.49 | 0.625 | s(day):treatmentTNCL | 2.350 | 2.914 | 4.514 | 0.006 ** |
| Residuals | 1194717 | 80 | | | | | | | | | | | |

## Dynamics of total algae

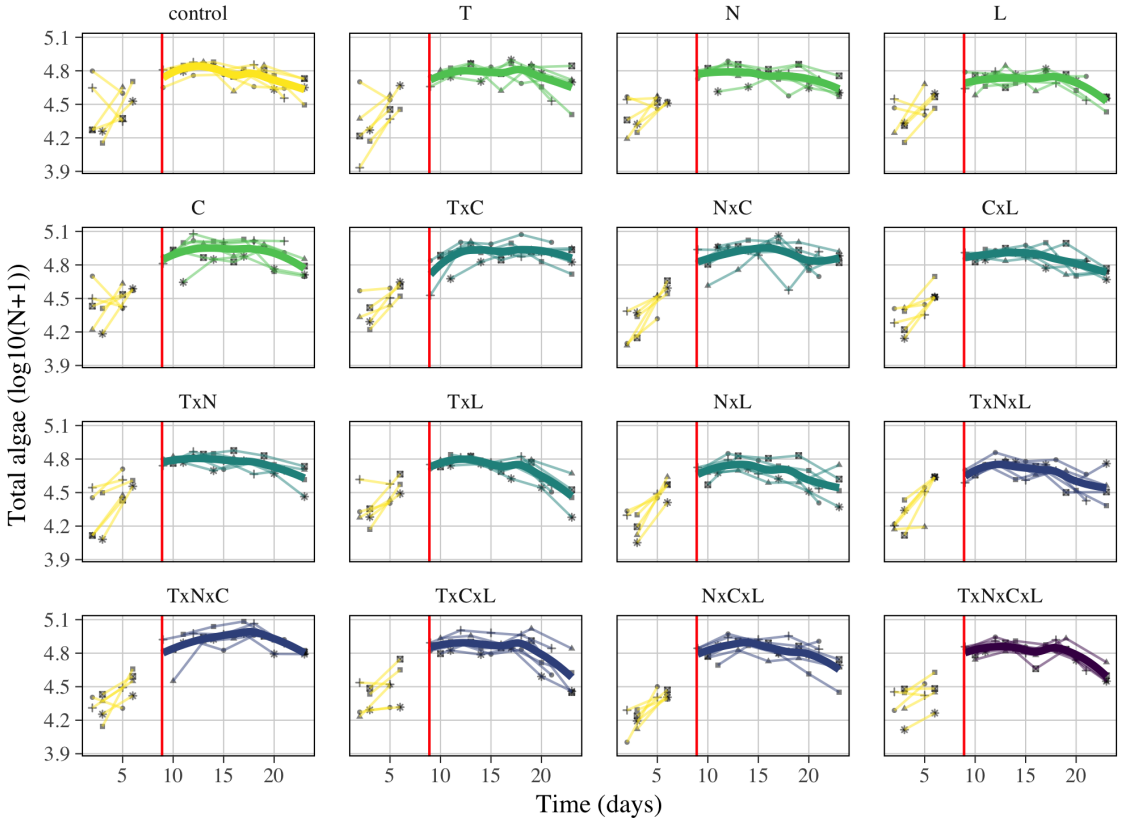



*Figure S10: Time serie of the abundance of total algae. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S8: Statisitcal analysis of the total algae densities. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 51.7% of deviance (Adj. $R^2$ = 47.9%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 123.342 | 1 | 9608.97 | <0.001 *** | -0.001 | 0.016 | -0.08 | 0.936 | s(day):treatmentno.change | 1.000 | 1.000 | 0.029 | 0.864 |
| Temperature (T) | 0.002 | 1 | 0.12 | 0.727 | 0.000 | 0.022 | 0.01 | 0.996 | s(day):treatmentT | 1.000 | 1.000 | 1.458 | 0.228 |
| Nutrient (N) | 0.000 | 1 | 0.03 | 0.874 | -0.009 | 0.022 | -0.42 | 0.671 | s(day):treatmentN | 1.000 | 1.000 | 0.271 | 0.603 |
| Carbon (C) | 0.000 | 1 | 0.01 | 0.926 | 0.145 | 0.022 | 6.50 | <0.001 *** | s(day):treatmentC | 1.061 | 1.120 | 1.704 | 0.207 |
| Light (L) | 0.000 | 1 | 0.01 | 0.921 | -0.063 | 0.022 | -2.83 | 0.005 ** | s(day):treatmentL | 1.176 | 1.331 | 0.169 | 0.818 |
| T:N | 0.006 | 1 | 0.50 | 0.483 | 0.010 | 0.032 | 0.31 | 0.757 | s(day):treatmentTN | 1.000 | 1.000 | 0.018 | 0.895 |
| T:C | 0.003 | 1 | 0.26 | 0.609 | -0.008 | 0.032 | -0.25 | 0.807 | s(day):treatmentTC | 1.000 | 1.001 | 21.198 | <0.001 *** |
| N:C | 0.000 | 1 | 0.01 | 0.937 | -0.005 | 0.032 | -0.17 | 0.865 | s(day):treatmentNC | 1.000 | 1.000 | 6.801 | 0.009 ** |
| T:L | 0.001 | 1 | 0.04 | 0.834 | 0.006 | 0.032 | 0.19 | 0.847 | s(day):treatmentTL | 1.503 | 1.846 | 3.119 | 0.033 * |
| N:L | 0.000 | 1 | 0.00 | 0.956 | -0.013 | 0.032 | -0.41 | 0.679 | s(day):treatmentNL | 1.000 | 1.000 | 0.142 | 0.707 |
| C:L | 0.000 | 1 | 0.03 | 0.865 | 0.014 | 0.032 | 0.45 | 0.653 | s(day):treatmentCL | 1.000 | 1.000 | 0.020 | 0.888 |
| T:N:C | 0.009 | 1 | 0.73 | 0.396 | 0.016 | 0.045 | 0.35 | 0.725 | s(day):treatmentTNC | 2.020 | 2.512 | 5.113 | 0.005 ** |
| T:N:L | 0.002 | 1 | 0.19 | 0.664 | -0.019 | 0.045 | -0.42 | 0.672 | s(day):treatmentTNL | 1.000 | 1.000 | 0.317 | 0.574 |
| T:C:L | 0.001 | 1 | 0.09 | 0.763 | -0.031 | 0.045 | -0.70 | 0.487 | s(day):treatmentTCL | 2.161 | 2.684 | 2.667 | 0.084 |
| N:C:L | 0.004 | 1 | 0.31 | 0.579 | -0.014 | 0.045 | -0.30 | 0.762 | s(day):treatmentNCL | 1.000 | 1.000 | 0.010 | 0.921 |
| T:N:C:L | 0.006 | 1 | 0.46 | 0.498 | 0.008 | 0.063 | 0.13 | 0.897 | s(day):treatmentTNCL | 1.452 | 1.772 | 0.344 | 0.587 |
| Residuals | 1.027 | 80 | | | | | | | | | | | |

## Dynamics of total bacteria

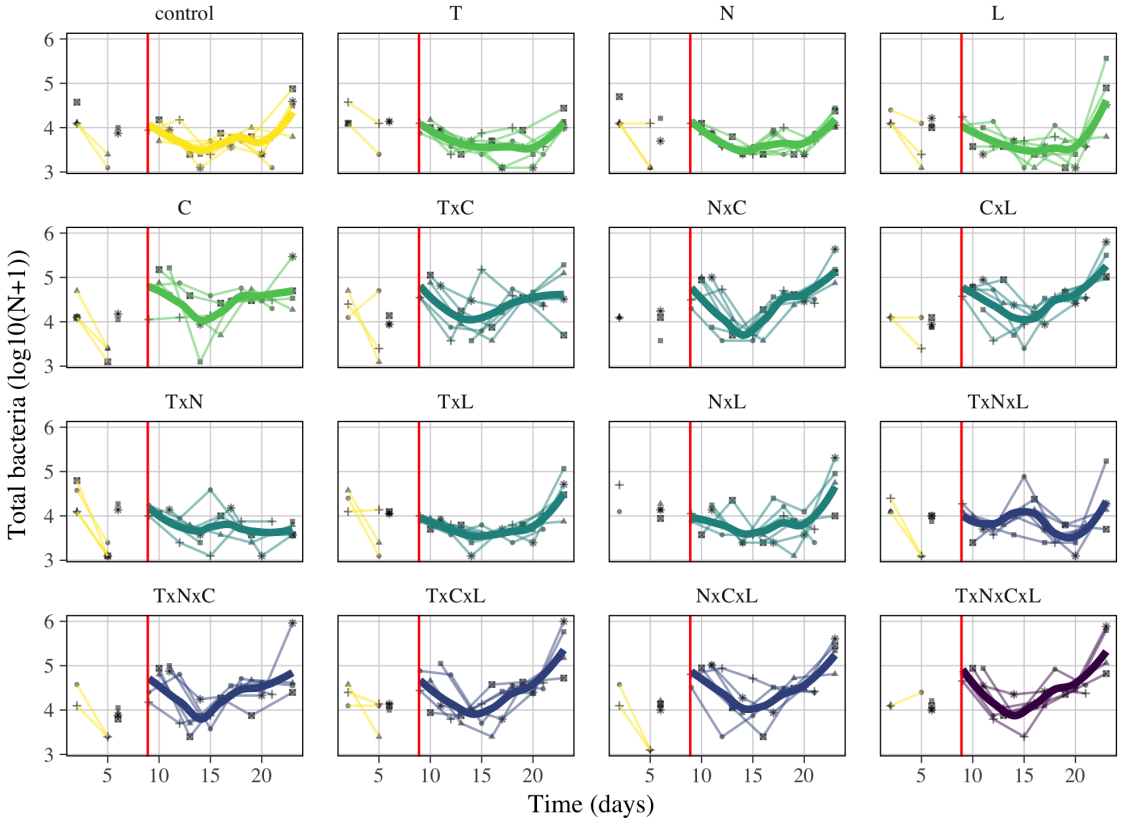



*Figure S11: Time serie of the abundance of total bacteria. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line; **T**: 25°C, **N**: N:P=15, **C**: 0.56g.L$^{-1}$ PPM, and **L**: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S9: Statisitcal analysis of the total bacteria densities. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 54.5% of deviance (Adj. $R^2$ = 50%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 51.628 | 1 | 291.68 | <0.001 *** | -0.013 | 0.064 | -0.21 | 0.834 | s(day):treatmentno.change | 1.000 | 1.000 | 0.143 | 0.706 |
| Temperature (T) | 0.245 | 1 | 1.39 | 0.243 | -0.072 | 0.090 | -0.79 | 0.428 | s(day):treatmentT | 1.000 | 1.000 | 2.687 | 0.102 |
| Nutrient (N) | 0.005 | 1 | 0.03 | 0.866 | -0.044 | 0.090 | -0.49 | 0.624 | s(day):treatmentN | 1.000 | 1.000 | 1.109 | 0.293 |
| Carbon (C) | 0.002 | 1 | 0.01 | 0.911 | 0.698 | 0.090 | 7.73 | <0.001 *** | s(day):treatmentC | 1.000 | 1.000 | 1.433 | 0.232 |
| Light (L) | 0.112 | 1 | 0.64 | 0.428 | -0.034 | 0.090 | -0.38 | 0.705 | s(day):treatmentL | 2.631 | 3.257 | 1.376 | 0.225 |
| T:N | 0.259 | 1 | 1.46 | 0.231 | 0.077 | 0.130 | 0.60 | 0.550 | s(day):treatmentTN | 2.134 | 2.638 | 5.871 | 0.001 ** |
| T:C | 0.013 | 1 | 0.07 | 0.790 | -0.025 | 0.128 | -0.19 | 0.847 | s(day):treatmentTC | 1.000 | 1.000 | 0.411 | 0.522 |
| N:C | 0.091 | 1 | 0.52 | 0.475 | -0.041 | 0.128 | -0.32 | 0.747 | s(day):treatmentNC | 2.565 | 3.176 | 2.064 | 0.091 |
| T:L | 0.150 | 1 | 0.85 | 0.360 | 0.122 | 0.128 | 0.96 | 0.339 | s(day):treatmentTL | 1.000 | 1.000 | 0.440 | 0.508 |
| N:L | 0.082 | 1 | 0.46 | 0.499 | 0.182 | 0.128 | 1.42 | 0.156 | s(day):treatmentNL | 1.000 | 1.000 | 0.739 | 0.390 |
| C:L | 0.002 | 1 | 0.01 | 0.915 | 0.071 | 0.128 | 0.55 | 0.580 | s(day):treatmentCL | 1.679 | 2.085 | 1.159 | 0.313 |
| T:N:C | 0.000 | 1 | 0.00 | 0.999 | 0.009 | 0.182 | 0.05 | 0.959 | s(day):treatmentTNC | 1.000 | 1.000 | 0.037 | 0.847 |
| T:N:L | 0.001 | 1 | 0.00 | 0.953 | -0.142 | 0.182 | -0.78 | 0.435 | s(day):treatmentTNL | 4.405 | 5.371 | 2.942 | 0.012 * |
| T:C:L | 0.028 | 1 | 0.16 | 0.694 | -0.117 | 0.181 | -0.65 | 0.518 | s(day):treatmentTCL | 1.986 | 2.470 | 3.523 | 0.021 * |
| N:C:L | 0.300 | 1 | 1.69 | 0.198 | -0.112 | 0.181 | -0.62 | 0.536 | s(day):treatmentNCL | 2.039 | 2.535 | 1.256 | 0.276 |
| T:N:C:L | 0.187 | 1 | 1.06 | 0.308 | 0.112 | 0.257 | 0.44 | 0.662 | s(day):treatmentTNCL | 2.216 | 2.752 | 2.353 | 0.060 |
| Residuals | 11.859 | 67 | | | | | | | | | | | |

## Dynamics of *Colpidium striatum*

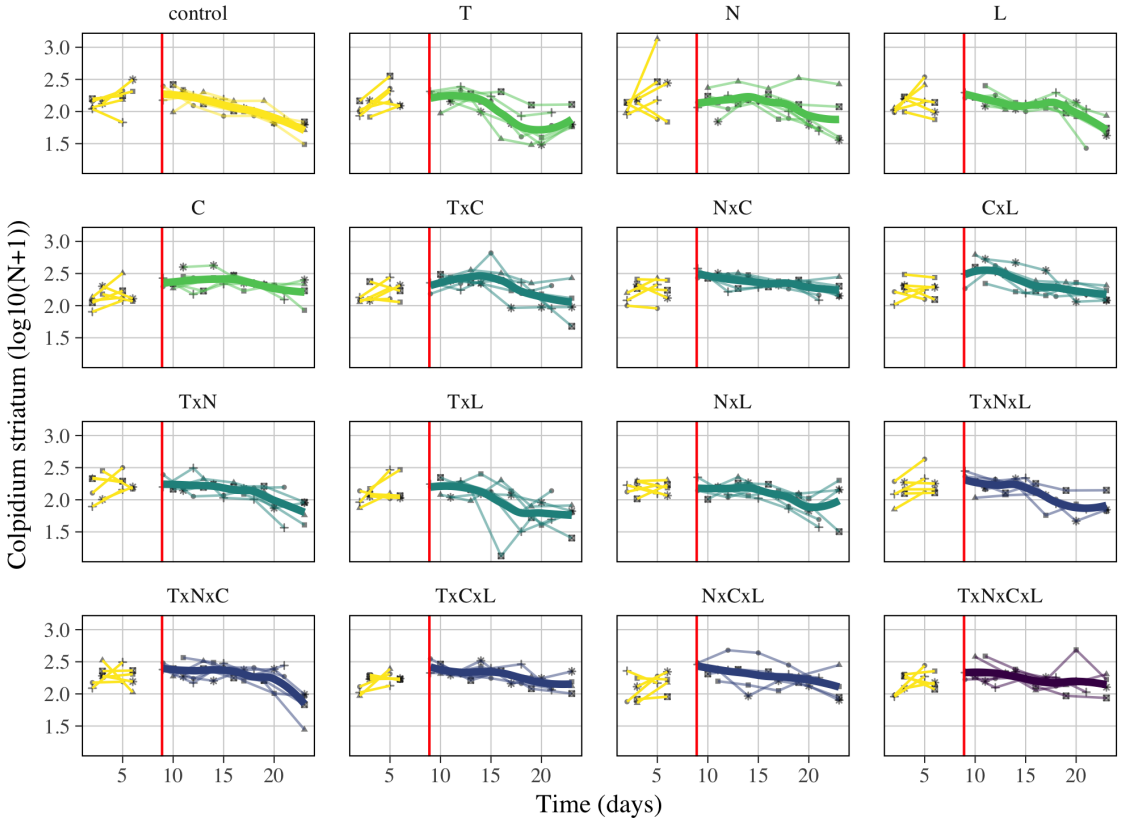



*Figure S12: Time serie of the abundance of Colpidium striatum. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S10: Statisitcal analysis of Colpidium striatum's densities. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 49.5% of deviance (Adj.* $R^2$ *= 44.9%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 29.894 | 1 | 735.52 | <0.001 *** | 0.000 | 0.030 | 0.01 | 0.994 | s(day):treatmentno.change | 1.000 | 1.000 | 0.001 | 0.977 |
| Temperature (T) | 0.003 | 1 | 0.06 | 0.802 | -0.048 | 0.042 | -1.14 | 0.255 | s(day):treatmentT | 3.609 | 4.435 | 3.211 | 0.010 * |
| Nutrient (N) | 0.024 | 1 | 0.58 | 0.448 | 0.033 | 0.042 | 0.78 | 0.435 | s(day):treatmentN | 1.000 | 1.000 | 6.125 | 0.014 * |
| Carbon (C) | 0.006 | 1 | 0.15 | 0.698 | 0.294 | 0.042 | 6.97 | <0.001 *** | s(day):treatmentC | 1.000 | 1.000 | 16.578 | <0.001 *** |
| Light (L) | 0.007 | 1 | 0.17 | 0.681 | 0.013 | 0.042 | 0.31 | 0.754 | s(day):treatmentL | 1.000 | 1.000 | 1.059 | 0.304 |
| T:N | 0.011 | 1 | 0.27 | 0.602 | 0.073 | 0.060 | 1.22 | 0.225 | s(day):treatmentTN | 1.000 | 1.000 | 1.821 | 0.178 |
| T:C | 0.001 | 1 | 0.02 | 0.896 | -0.005 | 0.060 | -0.08 | 0.939 | s(day):treatmentTC | 1.467 | 1.794 | 3.234 | 0.076 |
| N:C | 0.002 | 1 | 0.04 | 0.849 | -0.019 | 0.060 | -0.32 | 0.749 | s(day):treatmentNC | 1.188 | 1.352 | 8.162 | 0.001 ** |
| T:L | 0.001 | 1 | 0.02 | 0.888 | -0.028 | 0.060 | -0.47 | 0.639 | s(day):treatmentTL | 1.983 | 2.467 | 0.968 | 0.538 |
| N:L | 0.011 | 1 | 0.28 | 0.599 | -0.023 | 0.060 | -0.39 | 0.700 | s(day):treatmentNL | 2.971 | 3.671 | 2.746 | 0.023 * |
| C:L | 0.016 | 1 | 0.40 | 0.527 | 0.006 | 0.060 | 0.10 | 0.921 | s(day):treatmentCL | 1.528 | 1.881 | 1.213 | 0.228 |
| T:N:C | 0.002 | 1 | 0.05 | 0.820 | -0.110 | 0.085 | -1.30 | 0.193 | s(day):treatmentTNC | 2.275 | 2.822 | 2.194 | 0.136 |
| T:N:L | 0.020 | 1 | 0.49 | 0.487 | 0.020 | 0.085 | 0.24 | 0.815 | s(day):treatmentTNL | 1.527 | 1.879 | 0.659 | 0.425 |
| T:C:L | 0.000 | 1 | 0.01 | 0.934 | 0.000 | 0.085 | 0.00 | 0.998 | s(day):treatmentTCL | 1.000 | 1.000 | 10.412 | 0.001 ** |
| N:C:L | 0.000 | 1 | 0.01 | 0.923 | -0.069 | 0.085 | -0.82 | 0.412 | s(day):treatmentNCL | 1.000 | 1.000 | 8.714 | 0.003 ** |
| T:N:C:L | 0.003 | 1 | 0.07 | 0.797 | 0.059 | 0.120 | 0.50 | 0.619 | s(day):treatmentTNCL | 1.464 | 1.789 | 7.507 | <0.001 *** |
| Residuals | 3.252 | 80 | | | | | | | | | | | |

## Dynamics of *Tetrahymena thermophila*

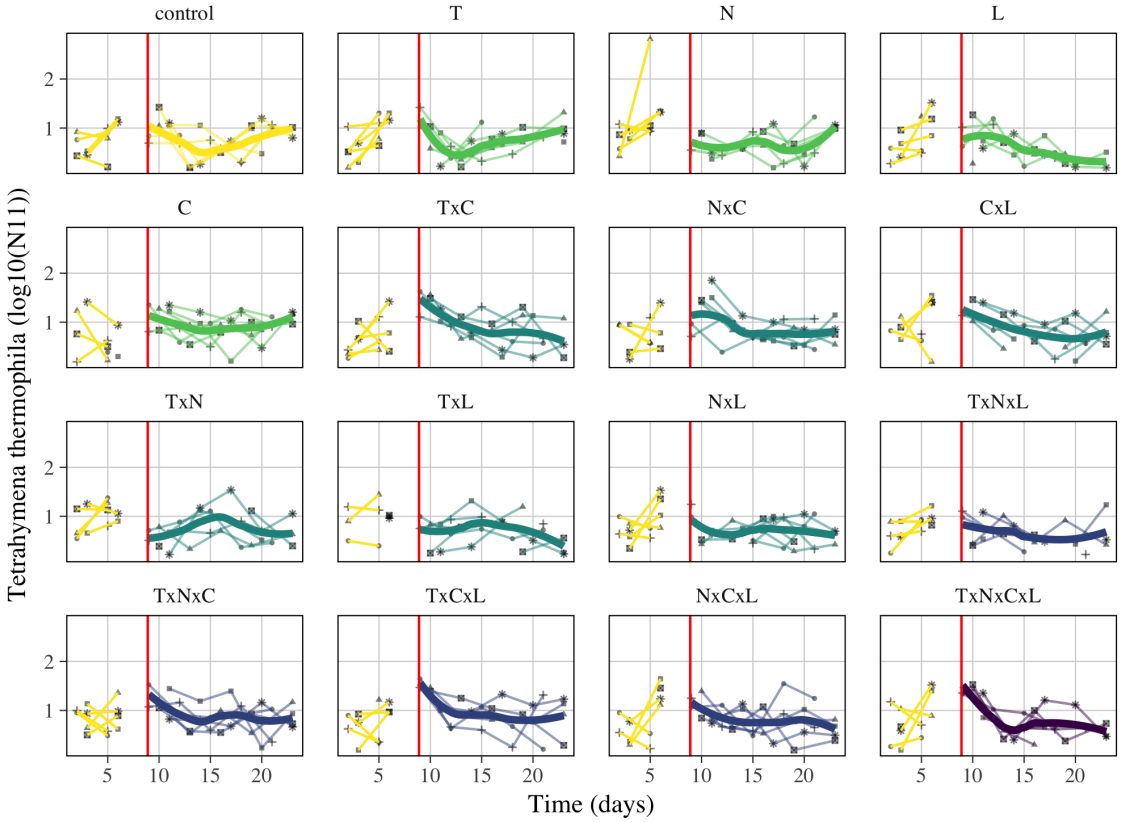



*Figure S13: Time serie of the abundance of Tetrahymena thermophila. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S11: Statisitcal analysis of Tetrahymena thermophila's densities. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 38.8% of deviance (Adj. $R^2$ = 31.7%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 4.529 | 1 | 27.46 | <0.001 *** | 0.013 | 0.055 | 0.24 | 0.809 | s(day):treatmentno.change | 1.000 | 1.000 | 0.001 | 0.979 |
| Temperature (T) | 0.096 | 1 | 0.58 | 0.447 | -0.030 | 0.079 | -0.38 | 0.706 | s(day):treatmentT | 1.000 | 1.000 | 0.413 | 0.521 |
| Nutrient (N) | 0.855 | 1 | 5.18 | 0.026 * | -0.100 | 0.079 | -1.27 | 0.205 | s(day):treatmentN | 3.113 | 3.858 | 1.401 | 0.176 |
| Carbon (C) | 0.419 | 1 | 2.54 | 0.115 | 0.171 | 0.076 | 2.24 | 0.026 * | s(day):treatmentC | 1.140 | 1.267 | 0.180 | 0.657 |
| Light (L) | 0.029 | 1 | 0.18 | 0.675 | -0.211 | 0.082 | -2.59 | 0.010 * | s(day):treatmentL | 2.615 | 3.239 | 4.767 | 0.002 ** |
| T:N | 0.363 | 1 | 2.20 | 0.142 | 0.083 | 0.112 | 0.74 | 0.458 | s(day):treatmentTN | 3.563 | 4.386 | 6.232 | <0.001 *** |
| T:C | 0.021 | 1 | 0.13 | 0.722 | -0.001 | 0.109 | -0.01 | 0.990 | s(day):treatmentTC | 1.587 | 1.966 | 8.829 | <0.001 *** |
| N:C | 0.070 | 1 | 0.43 | 0.516 | 0.051 | 0.108 | 0.47 | 0.638 | s(day):treatmentNC | 1.917 | 2.385 | 4.574 | 0.006 ** |
| T:L | 0.036 | 1 | 0.22 | 0.642 | 0.166 | 0.114 | 1.46 | 0.146 | s(day):treatmentTL | 3.044 | 3.763 | 6.284 | <0.001 *** |
| N:L | 0.381 | 1 | 2.31 | 0.132 | 0.240 | 0.113 | 2.12 | 0.035 * | s(day):treatmentNL | 2.410 | 2.982 | 2.626 | 0.051 |
| C:L | 0.234 | 1 | 1.42 | 0.238 | 0.128 | 0.111 | 1.15 | 0.250 | s(day):treatmentCL | 1.405 | 1.704 | 6.188 | 0.003 ** |
| T:N:C | 0.030 | 1 | 0.18 | 0.670 | -0.042 | 0.153 | -0.28 | 0.783 | s(day):treatmentTNC | 1.654 | 2.052 | 3.811 | 0.022 * |
| T:N:L | 0.114 | 1 | 0.69 | 0.409 | -0.285 | 0.161 | -1.77 | 0.078 | s(day):treatmentTNL | 1.895 | 2.349 | 1.716 | 0.212 |
| T:C:L | 0.065 | 1 | 0.39 | 0.533 | -0.037 | 0.156 | -0.24 | 0.812 | s(day):treatmentTCL | 1.000 | 1.000 | 10.371 | 0.001 ** |
| N:C:L | 0.040 | 1 | 0.24 | 0.623 | -0.240 | 0.155 | -1.55 | 0.123 | s(day):treatmentNCL | 2.134 | 2.650 | 4.059 | 0.012 * |
| T:N:C:L | 0.000 | 1 | 0.00 | 0.988 | 0.154 | 0.221 | 0.70 | 0.487 | s(day):treatmentTNCL | 1.000 | 1.000 | 15.432 | <0.001 *** |
| Residuals | 13.028 | 79 | | | | | | | | | | | |

## Dynamics of *Paramecium*

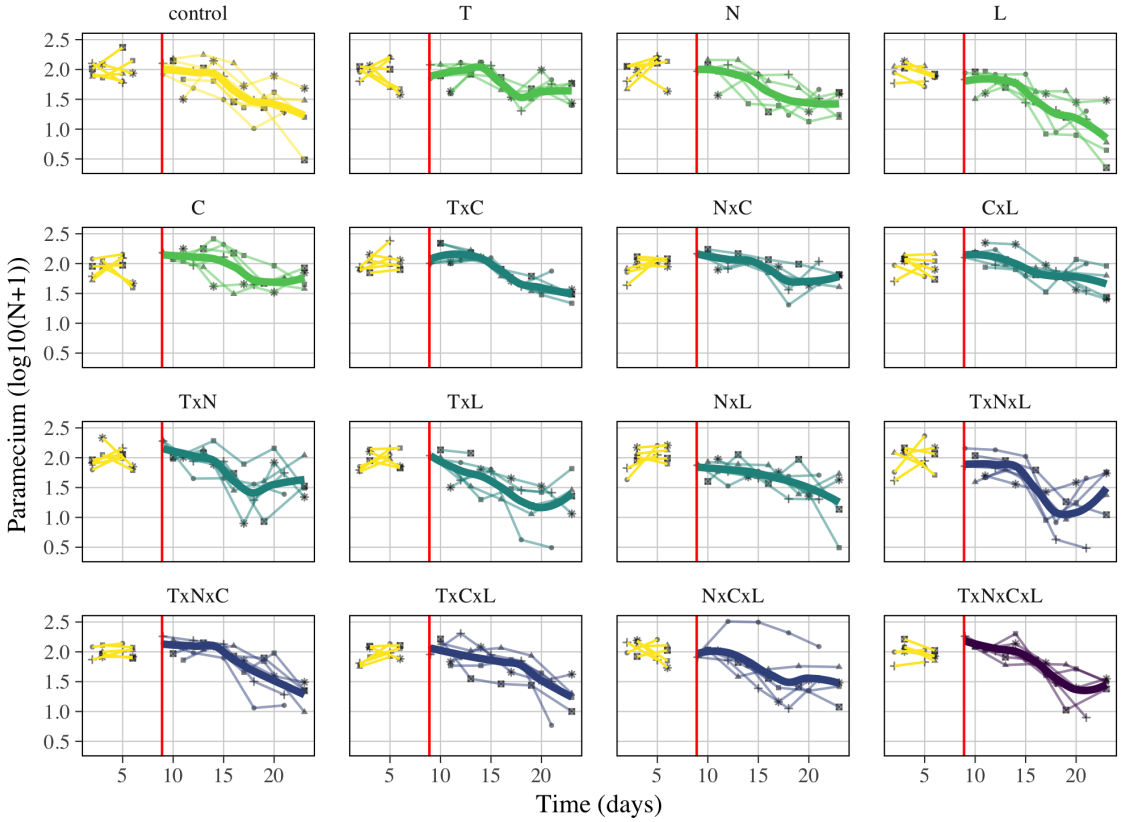



*Figure S14: Time serie of the abundance of Paramecium. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S12: Statistical analysis of Paramecium's densities. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e., **Pre-perturbation**). The time series were analysed with a GAM explaining 34.8% of deviance (Adj. $R^2$ = 29.3%), with the main effects and their interactions as **parametric coefficients** and the temporal change with a spline function the for each treatment (i.e., **smooth term**).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 23.864 | 1 | 806.68 | <0.001 *** | 0.002 | 0.044 | 0.04 | 0.965 | s(day):treatmentno.change | 1.000 | 1.000 | 0.059 | 0.809 |
| Temperature (T) | 0.033 | 1 | 1.13 | 0.291 | 0.127 | 0.062 | 2.04 | 0.042 * | s(day):treatmentT | 1.000 | 1.000 | 12.146 | <0.001 *** |
| Nutrient (N) | 0.020 | 1 | 0.66 | 0.419 | 0.020 | 0.062 | 0.32 | 0.751 | s(day):treatmentN | 1.137 | 1.261 | 1.032 | 0.263 |
| Carbon (C) | 0.011 | 1 | 0.37 | 0.546 | 0.258 | 0.062 | 4.15 | <0.001 *** | s(day):treatmentC | 1.000 | 1.000 | 6.843 | 0.009 ** |
| Light (L) | 0.043 | 1 | 1.44 | 0.233 | -0.205 | 0.062 | -3.30 | 0.001 ** | s(day):treatmentL | 1.000 | 1.000 | 1.595 | 0.207 |
| T:N | 0.000 | 1 | 0.01 | 0.936 | -0.039 | 0.088 | -0.44 | 0.658 | s(day):treatmentTN | 2.341 | 2.903 | 3.126 | 0.028 * |
| T:C | 0.081 | 1 | 2.75 | 0.101 | -0.193 | 0.088 | -2.20 | 0.028 * | s(day):treatmentTC | 1.000 | 1.000 | 0.683 | 0.409 |
| N:C | 0.000 | 1 | 0.01 | 0.932 | -0.032 | 0.088 | -0.36 | 0.720 | s(day):treatmentNC | 1.226 | 1.417 | 5.463 | 0.008 ** |
| T:L | 0.081 | 1 | 2.75 | 0.101 | -0.050 | 0.088 | -0.57 | 0.573 | s(day):treatmentTL | 2.430 | 3.012 | 2.066 | 0.102 |
| N:L | 0.023 | 1 | 0.77 | 0.383 | 0.155 | 0.088 | 1.77 | 0.078 | s(day):treatmentNL | 1.000 | 1.000 | 4.551 | 0.033 * |
| C:L | 0.022 | 1 | 0.74 | 0.392 | 0.194 | 0.088 | 2.20 | 0.028 * | s(day):treatmentCL | 1.000 | 1.000 | 6.054 | 0.014 * |
| T:N:C | 0.018 | 1 | 0.61 | 0.439 | -0.002 | 0.124 | -0.02 | 0.987 | s(day):treatmentTNC | 1.001 | 1.002 | 0.103 | 0.749 |
| T:N:L | 0.030 | 1 | 1.00 | 0.321 | -0.133 | 0.125 | -1.07 | 0.286 | s(day):treatmentTNL | 3.618 | 4.445 | 3.094 | 0.011 * |
| T:C:L | 0.060 | 1 | 2.01 | 0.160 | -0.056 | 0.125 | -0.45 | 0.652 | s(day):treatmentTCL | 1.405 | 1.703 | 0.414 | 0.712 |
| N:C:L | 0.026 | 1 | 0.89 | 0.348 | -0.328 | 0.124 | -2.64 | 0.009 ** | s(day):treatmentNCL | 1.279 | 1.506 | 2.138 | 0.091 |
| T:N:C:L | 0.020 | 1 | 0.67 | 0.414 | 0.383 | 0.176 | 2.18 | 0.030 * | s(day):treatmentTNCL | 1.000 | 1.000 | 0.026 | 0.871 |
| Residuals | 2.367 | 80 | | | | | | | | | | | |

## Dynamics of *Paramecium bursaria*

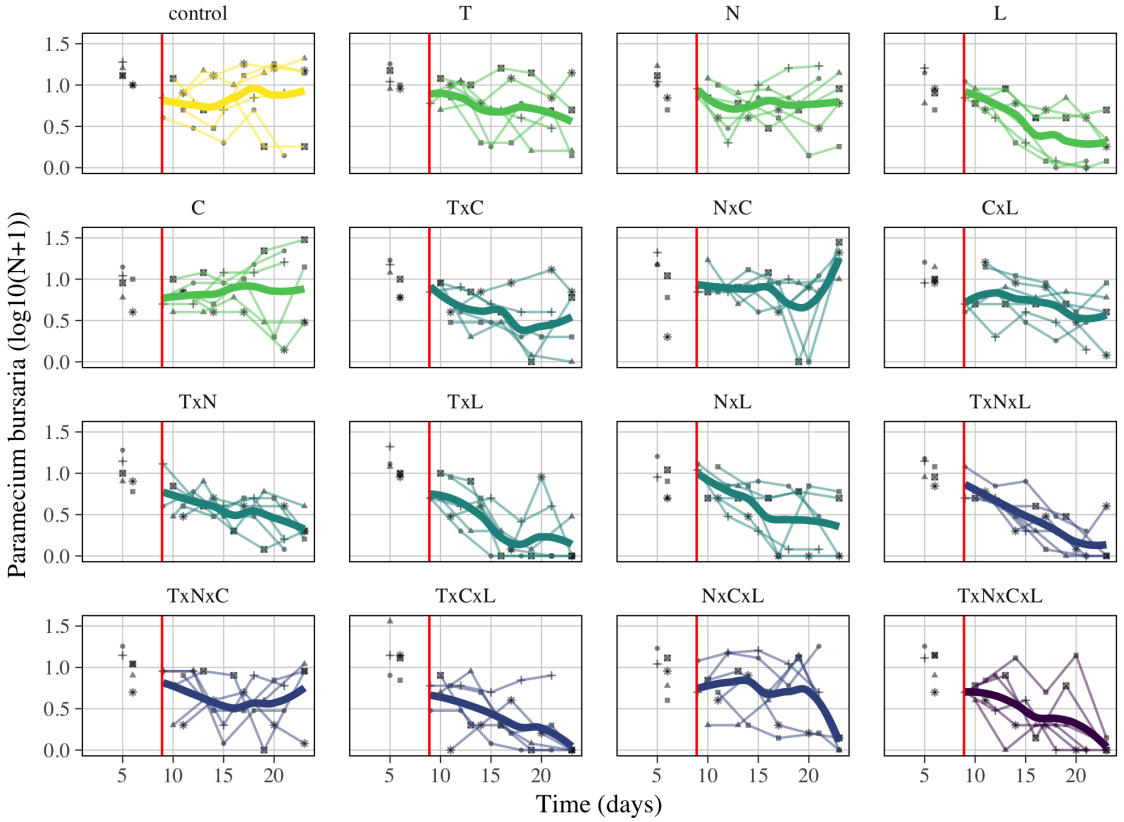



*Figure S15: Time serie of the abundance of Paramecium bursaria. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S13: Statisitcal analysis of Paramecium bursaria's densities. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e., **Pre-perturbation**). The time series were analysed with a GAM explaining 46.7% of deviance (Adj. $R^2$ = 42.2%), with the main effects and their interactions as **parametric coefficients** and the temporal change with a spline function the for each treatment (i.e., **smooth term**).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 7.506 | 1 | 189.69 | <0.001 *** | -0.004 | 0.053 | -0.08 | 0.934 | s(day):treatmentno.change | 1.000 | 1.000 | 0.047 | 0.828 |
| Temperature (T) | 0.009 | 1 | 0.23 | 0.634 | -0.101 | 0.075 | -1.35 | 0.179 | s(day):treatmentT | 1.000 | 1.000 | 9.603 | 0.002 ** |
| Nutrient (N) | 0.051 | 1 | 1.28 | 0.260 | -0.062 | 0.075 | -0.83 | 0.409 | s(day):treatmentN | 1.000 | 1.000 | 1.540 | 0.215 |
| Carbon (C) | 0.118 | 1 | 2.98 | 0.088 | 0.006 | 0.075 | 0.08 | 0.939 | s(day):treatmentC | 1.000 | 1.000 | 0.285 | 0.594 |
| Light (L) | 0.088 | 1 | 2.22 | 0.140 | -0.308 | 0.075 | -4.11 | <0.001 *** | s(day):treatmentL | 1.084 | 1.163 | 23.990 | <0.001 *** |
| T:N | 0.007 | 1 | 0.18 | 0.676 | -0.134 | 0.106 | -1.26 | 0.207 | s(day):treatmentTN | 1.000 | 1.000 | 12.805 | <0.001 *** |
| T:C | 0.030 | 1 | 0.76 | 0.386 | -0.151 | 0.106 | -1.43 | 0.155 | s(day):treatmentTC | 1.274 | 1.496 | 7.889 | 0.004 ** |
| N:C | 0.046 | 1 | 1.17 | 0.283 | 0.102 | 0.106 | 0.96 | 0.338 | s(day):treatmentNC | 3.356 | 4.133 | 2.549 | 0.037 * |
| T:L | 0.052 | 1 | 1.31 | 0.256 | -0.043 | 0.106 | -0.40 | 0.687 | s(day):treatmentTL | 1.491 | 1.828 | 14.927 | <0.001 *** |
| N:L | 0.015 | 1 | 0.38 | 0.542 | 0.119 | 0.106 | 1.12 | 0.264 | s(day):treatmentNL | 1.000 | 1.000 | 22.427 | <0.001 *** |
| C:L | 0.130 | 1 | 3.27 | 0.074 | 0.151 | 0.106 | 1.43 | 0.155 | s(day):treatmentCL | 1.000 | 1.000 | 8.968 | 0.003 ** |
| T:N:C | 0.009 | 1 | 0.21 | 0.645 | 0.137 | 0.150 | 0.92 | 0.361 | s(day):treatmentTNC | 1.875 | 2.334 | 2.194 | 0.126 |
| T:N:L | 0.006 | 1 | 0.15 | 0.696 | 0.129 | 0.150 | 0.86 | 0.391 | s(day):treatmentTNL | 1.000 | 1.000 | 33.187 | <0.001 *** |
| T:C:L | 0.029 | 1 | 0.74 | 0.393 | -0.004 | 0.150 | -0.03 | 0.978 | s(day):treatmentTCL | 1.000 | 1.000 | 22.071 | <0.001 *** |
| N:C:L | 0.041 | 1 | 1.04 | 0.310 | -0.185 | 0.150 | -1.23 | 0.219 | s(day):treatmentNCL | 2.687 | 3.329 | 6.660 | <0.001 *** |
| T:N:C:L | 0.008 | 1 | 0.19 | 0.660 | -0.056 | 0.212 | -0.27 | 0.791 | s(day):treatmentTNCL | 1.000 | 1.000 | 24.920 | <0.001 *** |
| Residuals | 3.166 | 80 | | | | | | | | | | | |

## Dynamics of *Blepharisma japonicum*

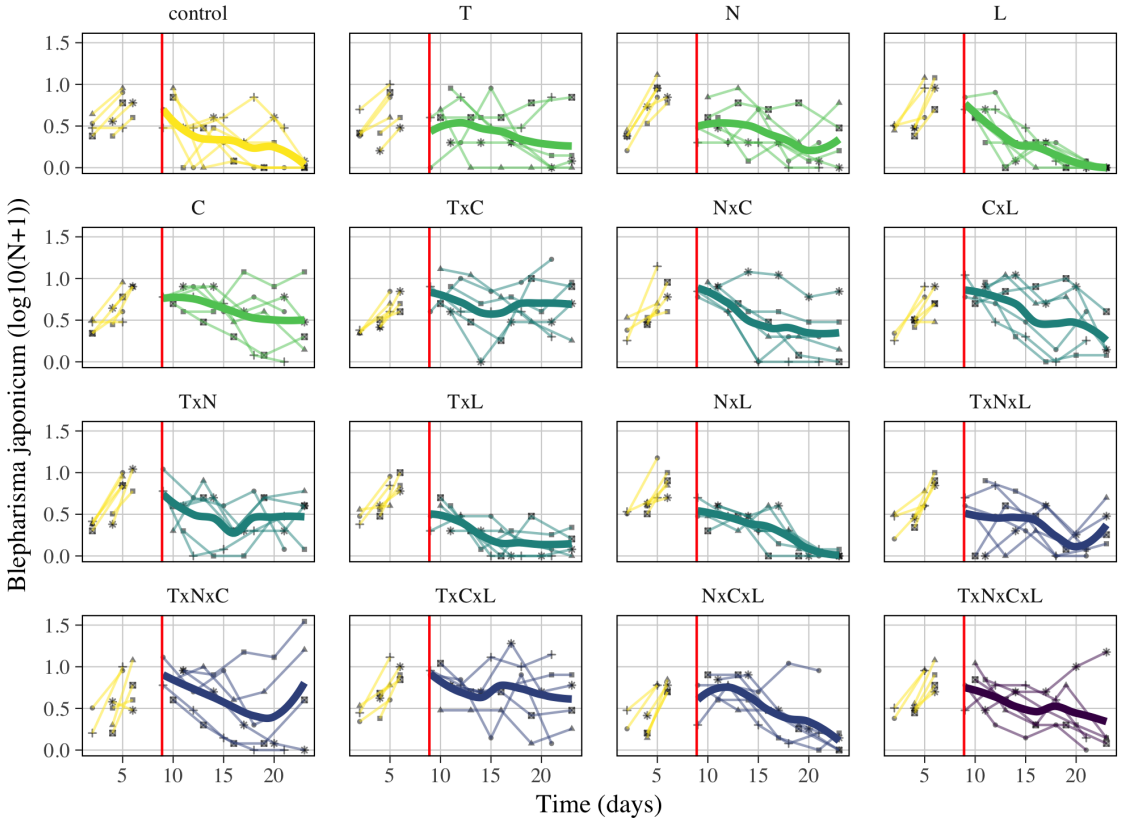



*Figure S16: Time serie of the abundance of Blepharisma japonicum. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S14: Statistical analysis of Belpharisma japonicum's densities. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e., **Pre-perturbation**). The time series were analysed with a GAM explaining 28% of deviance (Adj. $R^2$ = 22.2%), with the main effects and their interactions as **parametric coefficients** and the temporal change with a spline function the for each treatment (i.e., **smooth term**).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 3.364 | 1 | 119.52 | <0.001 *** | 0.001 | 0.051 | 0.02 | 0.987 | s(day):treatmentno.change | 1.000 | 1.000 | 0.030 | 0.862 |
| Temperature (T) | 0.000 | 1 | 0.01 | 0.913 | 0.089 | 0.073 | 1.23 | 0.219 | s(day):treatmentT | 1.000 | 1.000 | 1.617 | 0.204 |
| Nutrient (N) | 0.083 | 1 | 2.95 | 0.090 | 0.086 | 0.073 | 1.19 | 0.235 | s(day):treatmentN | 1.000 | 1.000 | 1.172 | 0.280 |
| Carbon (C) | 0.001 | 1 | 0.05 | 0.830 | 0.309 | 0.073 | 4.26 | <0.001 *** | s(day):treatmentC | 1.000 | 1.000 | 0.964 | 0.327 |
| Light (L) | 0.064 | 1 | 2.27 | 0.136 | -0.036 | 0.073 | -0.50 | 0.616 | s(day):treatmentL | 1.000 | 1.000 | 1.878 | 0.171 |
| T:N | 0.000 | 1 | 0.00 | 0.965 | -0.020 | 0.103 | -0.20 | 0.845 | s(day):treatmentTN | 1.295 | 1.531 | 2.495 | 0.066 |
| T:C | 0.003 | 1 | 0.10 | 0.751 | -0.018 | 0.103 | -0.18 | 0.860 | s(day):treatmentTC | 1.000 | 1.000 | 6.285 | 0.013 * |
| N:C | 0.029 | 1 | 1.03 | 0.313 | -0.193 | 0.103 | -1.88 | 0.061 | s(day):treatmentNC | 1.316 | 1.565 | 0.398 | 0.716 |
| T:L | 0.003 | 1 | 0.12 | 0.734 | -0.109 | 0.103 | -1.06 | 0.288 | s(day):treatmentTL | 1.000 | 1.000 | 0.374 | 0.541 |
| N:L | 0.045 | 1 | 1.61 | 0.208 | -0.075 | 0.103 | -0.73 | 0.467 | s(day):treatmentNL | 1.000 | 1.000 | 0.333 | 0.564 |
| C:L | 0.045 | 1 | 1.58 | 0.212 | -0.009 | 0.103 | -0.09 | 0.930 | s(day):treatmentCL | 1.000 | 1.000 | 0.301 | 0.584 |
| T:N:C | 0.003 | 1 | 0.12 | 0.733 | 0.056 | 0.145 | 0.39 | 0.699 | s(day):treatmentTNC | 3.034 | 3.746 | 2.914 | 0.021 * |
| T:N:L | 0.002 | 1 | 0.06 | 0.807 | 0.106 | 0.145 | 0.73 | 0.466 | s(day):treatmentTNL | 2.920 | 3.615 | 1.206 | 0.231 |
| T:C:L | 0.040 | 1 | 1.43 | 0.236 | 0.169 | 0.145 | 1.16 | 0.245 | s(day):treatmentTCL | 1.000 | 1.000 | 3.414 | 0.065 |
| N:C:L | 0.021 | 1 | 0.74 | 0.393 | 0.090 | 0.145 | 0.62 | 0.536 | s(day):treatmentNCL | 1.669 | 2.070 | 0.583 | 0.524 |
| T:N:C:L | 0.000 | 1 | 0.01 | 0.931 | -0.238 | 0.205 | -1.16 | 0.248 | s(day):treatmentTNCL | 1.000 | 1.000 | 0.484 | 0.487 |
| Residuals | 2.252 | 80 | | | | | | | | | | | |

## Dynamics of *Frontonia* sp.

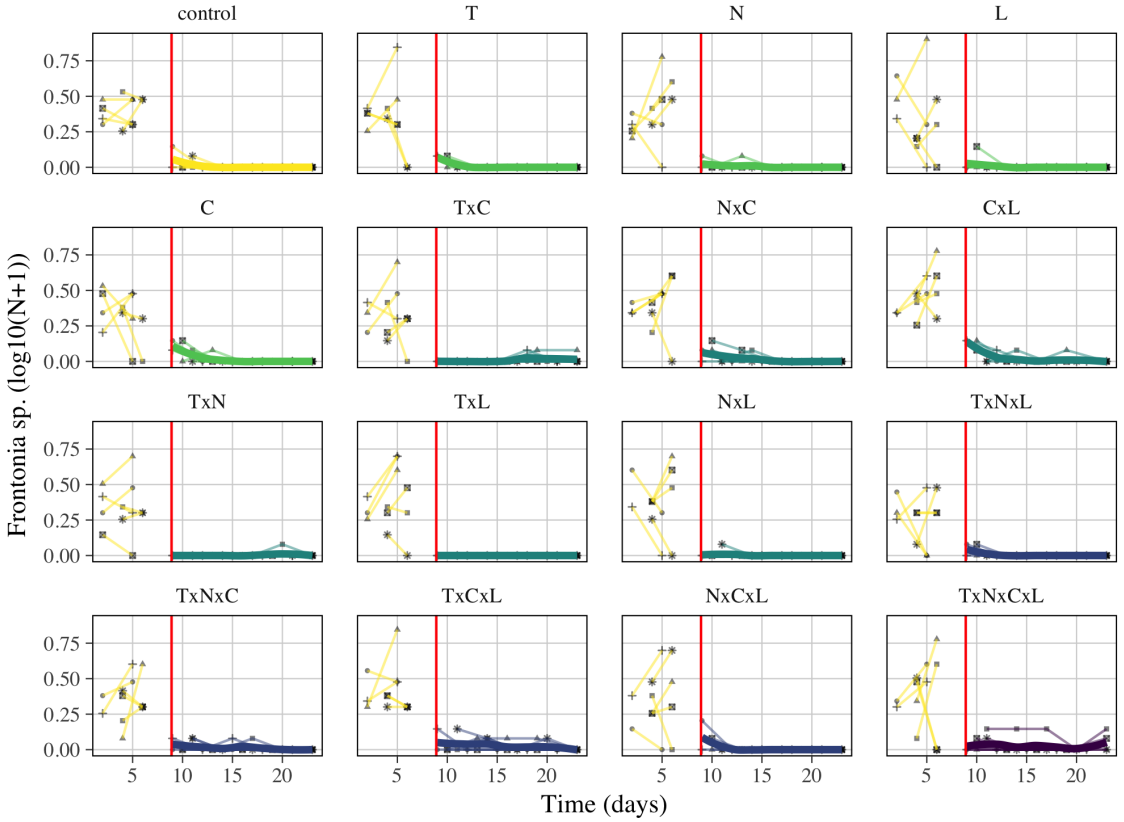



*Figure S17: Time serie of the abundance of Frontonia sp. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S15: Statisitcal analysis of Frontonia sp's densities. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 26.1% of deviance (Adj.* $R^2$ *= 18.5%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 1.051 | 1 | 16.59 | <0.001 *** | 0.000 | 0.005 | -0.08 | 0.940 | s(day):treatmentno.change | 1.000 | 1.000 | 0.006 | 0.938 |
| Temperature (T) | 0.029 | 1 | 0.45 | 0.503 | 0.000 | 0.007 | 0.06 | 0.954 | s(day):treatmentT | 1.000 | 1.000 | 0.027 | 0.870 |
| Nutrient (N) | 0.001 | 1 | 0.02 | 0.886 | -0.002 | 0.007 | -0.31 | 0.758 | s(day):treatmentN | 1.432 | 1.742 | 1.079 | 0.433 |
| Carbon (C) | 0.076 | 1 | 1.20 | 0.277 | 0.010 | 0.007 | 1.40 | 0.161 | s(day):treatmentC | 1.749 | 2.173 | 3.293 | 0.032 * |
| Light (L) | 0.023 | 1 | 0.37 | 0.546 | -0.003 | 0.007 | -0.37 | 0.711 | s(day):treatmentL | 1.000 | 1.001 | 0.775 | 0.379 |
| T:N | 0.000 | 1 | 0.00 | 0.981 | -0.003 | 0.010 | -0.30 | 0.766 | s(day):treatmentTN | 2.009 | 2.497 | 3.791 | 0.015 * |
| T:C | 0.051 | 1 | 0.81 | 0.371 | -0.010 | 0.010 | -0.99 | 0.321 | s(day):treatmentTC | 1.974 | 2.454 | 6.120 | 0.001 ** |
| N:C | 0.038 | 1 | 0.60 | 0.441 | 0.000 | 0.010 | 0.00 | 0.997 | s(day):treatmentNC | 1.000 | 1.000 | 1.555 | 0.213 |
| T:L | 0.080 | 1 | 1.26 | 0.266 | -0.005 | 0.010 | -0.51 | 0.609 | s(day):treatmentTL | 2.005 | 2.492 | 2.716 | 0.044 * |
| N:L | 0.000 | 1 | 0.00 | 0.982 | 0.000 | 0.010 | 0.00 | 0.997 | s(day):treatmentNL | 1.953 | 2.430 | 1.592 | 0.157 |
| C:L | 0.203 | 1 | 3.21 | 0.077 | 0.010 | 0.010 | 0.99 | 0.322 | s(day):treatmentCL | 2.879 | 3.559 | 4.952 | 0.002 ** |
| T:N:C | 0.008 | 1 | 0.12 | 0.730 | 0.011 | 0.014 | 0.73 | 0.466 | s(day):treatmentTNC | 1.773 | 2.205 | 0.597 | 0.513 |
| T:N:L | 0.038 | 1 | 0.60 | 0.441 | 0.011 | 0.014 | 0.73 | 0.468 | s(day):treatmentTNL | 1.000 | 1.000 | 0.432 | 0.511 |
| T:C:L | 0.124 | 1 | 1.96 | 0.165 | 0.015 | 0.014 | 1.07 | 0.286 | s(day):treatmentTCL | 2.070 | 2.572 | 1.072 | 0.296 |
| N:C:L | 0.093 | 1 | 1.47 | 0.229 | -0.013 | 0.014 | -0.93 | 0.354 | s(day):treatmentNCL | 1.006 | 1.013 | 0.412 | 0.528 |
| T:N:C:L | 0.078 | 1 | 1.24 | 0.270 | 0.000 | 0.020 | -0.02 | 0.988 | s(day):treatmentTNCL | 5.414 | 6.503 | 3.372 | 0.002 ** |
| Residuals | 5.066 | 80 | | | | | | | | | | | |

## Dynamics of *Rotifer* sp.

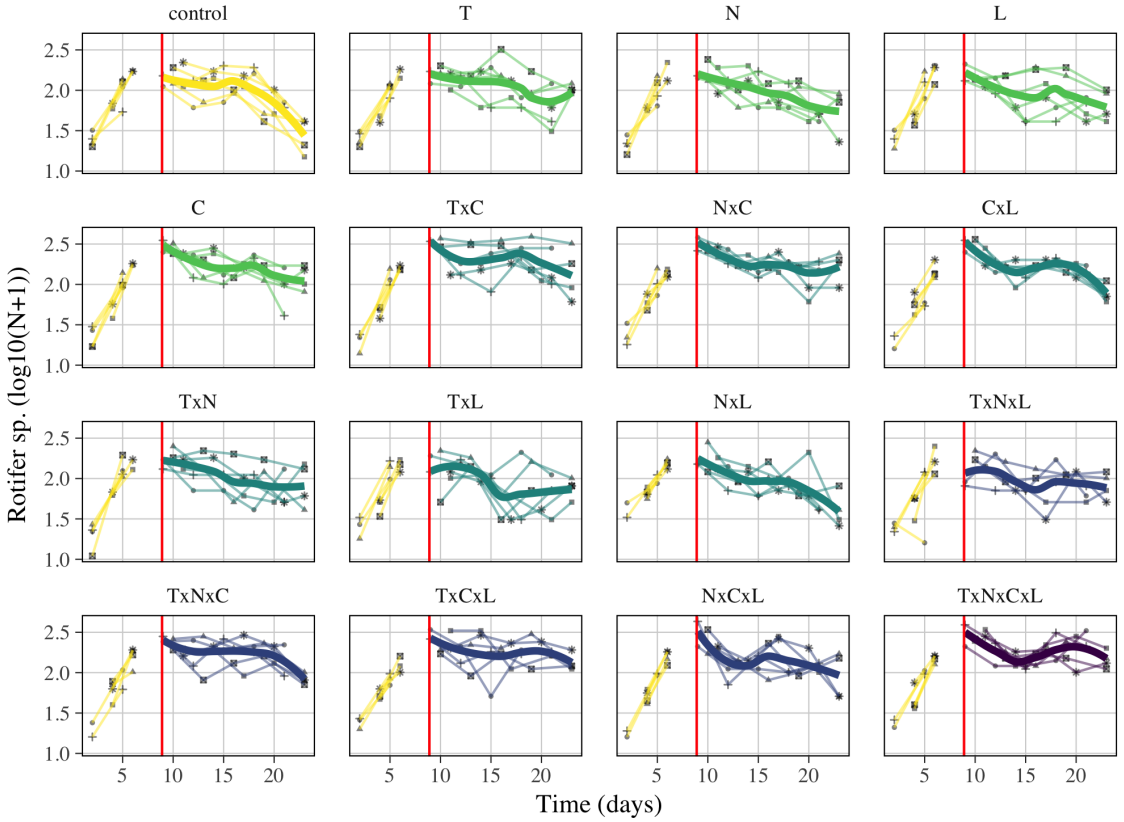



*Figure S18: Time serie of the abundance of Rotifer sp. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S16: Statisitcal analysis of Rotifer sp's densities. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 61.2% of deviance (Adj. $R^2$ = 55.6%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 25.981 | 1 | 865.47 | <0.001 *** | -0.001 | 0.032 | -0.02 | 0.987 | s(day):treatmentno.change | 1.000 | 1.000 | 0.000 | 0.989 |
| Temperature (T) | 0.000 | 1 | 0.00 | 0.988 | 0.099 | 0.046 | 2.14 | 0.033 * | s(day):treatmentT | 3.659 | 4.509 | 6.081 | <0.001 *** |
| Nutrient (N) | 0.000 | 1 | 0.00 | 0.975 | 0.014 | 0.046 | 0.31 | 0.757 | s(day):treatmentN | 2.827 | 3.501 | 3.615 | 0.009 ** |
| Carbon (C) | 0.001 | 1 | 0.04 | 0.836 | 0.265 | 0.046 | 5.77 | <0.001 *** | s(day):treatmentC | 2.830 | 3.506 | 5.360 | <0.001 *** |
| Light (L) | 0.014 | 1 | 0.45 | 0.505 | 0.025 | 0.046 | 0.54 | 0.589 | s(day):treatmentL | 2.724 | 3.378 | 5.878 | <0.001 *** |
| T:N | 0.005 | 1 | 0.17 | 0.683 | -0.036 | 0.065 | -0.56 | 0.579 | s(day):treatmentTN | 3.231 | 3.992 | 6.592 | <0.001 *** |
| T:C | 0.002 | 1 | 0.08 | 0.781 | -0.011 | 0.065 | -0.17 | 0.867 | s(day):treatmentTC | 2.395 | 2.971 | 6.190 | <0.001 *** |
| N:C | 0.001 | 1 | 0.02 | 0.897 | 0.037 | 0.065 | 0.57 | 0.569 | s(day):treatmentNC | 3.250 | 4.006 | 8.598 | <0.001 *** |
| T:L | 0.000 | 1 | 0.00 | 0.962 | -0.124 | 0.065 | -1.92 | 0.056 | s(day):treatmentTL | 4.726 | 5.741 | 7.387 | <0.001 *** |
| N:L | 0.001 | 1 | 0.03 | 0.859 | -0.041 | 0.065 | -0.63 | 0.527 | s(day):treatmentNL | 2.231 | 2.769 | 2.013 | 0.119 |
| C:L | 0.031 | 1 | 1.02 | 0.316 | -0.030 | 0.065 | -0.46 | 0.647 | s(day):treatmentCL | 2.584 | 3.200 | 3.810 | 0.009 ** |
| T:N:C | 0.004 | 1 | 0.14 | 0.709 | -0.096 | 0.092 | -1.04 | 0.299 | s(day):treatmentTNC | 1.848 | 2.300 | 4.259 | 0.011 * |
| T:N:L | 0.021 | 1 | 0.70 | 0.404 | 0.095 | 0.092 | 1.03 | 0.304 | s(day):treatmentTNL | 3.585 | 4.417 | 7.687 | <0.001 *** |
| T:C:L | 0.002 | 1 | 0.06 | 0.809 | 0.082 | 0.092 | 0.89 | 0.375 | s(day):treatmentTCL | 2.668 | 3.307 | 9.249 | <0.001 *** |
| N:C:L | 0.015 | 1 | 0.51 | 0.478 | -0.068 | 0.092 | -0.74 | 0.458 | s(day):treatmentNCL | 2.795 | 3.462 | 6.345 | <0.001 *** |
| T:N:C:L | 0.014 | 1 | 0.48 | 0.491 | 0.115 | 0.130 | 0.89 | 0.375 | s(day):treatmentTNCL | 3.048 | 3.763 | 10.790 | <0.001 *** |
| Residuals | 2.402 | 80 | | | | | | | | | | | |

## Dynamics of *Actinophrys sol*

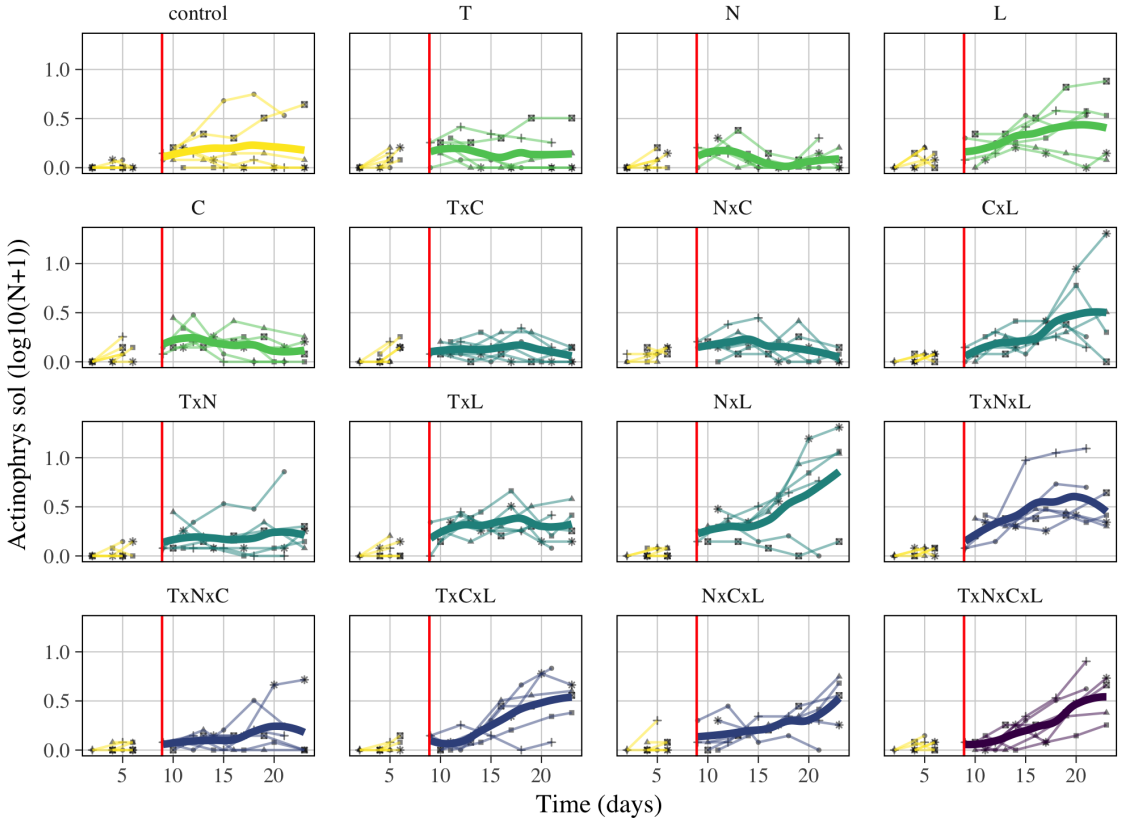



*Figure S19: Time serie of the abundance of Actinophrys sol. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S17: Statisitcal analysis of Actinophrys sol's densities. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 45.4% of deviance (Adj.* $R^2$ *= 40.1%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****). Due to differences between treatments in pre-perturbation, we added the mean pre-perturbation as random effect in the GAM.*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 0.001 | 1 | 0.20 | 0.653 | -0.001 | 0.032 | -0.04 | 0.965 | s(day):treatmentno.change | 1.000 | 1.000 | 0.177 | 0.674 |
| Temperature (T) | 0.033 | 1 | 6.52 | 0.013 * | -0.032 | 0.046 | -0.70 | 0.484 | s(day):treatmentT | 1.008 | 1.015 | 1.384 | 0.243 |
| Nutrient (N) | 0.015 | 1 | 2.99 | 0.087 | -0.087 | 0.046 | -1.91 | 0.057 | s(day):treatmentN | 1.659 | 2.057 | 1.794 | 0.174 |
| Carbon (C) | 0.033 | 1 | 6.38 | 0.014 * | 0.030 | 0.057 | 0.53 | 0.596 | s(day):treatmentC | 1.000 | 1.000 | 4.458 | 0.035 * |
| Light (L) | 0.033 | 1 | 6.52 | 0.013 * | 0.215 | 0.061 | 3.51 | < 0.001 *** | s(day):treatmentL | 1.000 | 1.000 | 4.892 | 0.027 * |
| T:N | 0.030 | 1 | 5.86 | 0.018 * | 0.098 | 0.067 | 1.47 | 0.144 | s(day):treatmentTN | 1.000 | 1.000 | 0.016 | 0.900 |
| T:C | 0.014 | 1 | 2.80 | 0.098 | -0.064 | 0.073 | -0.88 | 0.381 | s(day):treatmentTC | 1.000 | 1.000 | 0.989 | 0.321 |
| N:C | 0.015 | 1 | 2.98 | 0.088 | 0.035 | 0.077 | 0.45 | 0.652 | s(day):treatmentNC | 1.000 | 1.000 | 3.101 | 0.079 |
| T:L | 0.035 | 1 | 6.82 | 0.011 * | -0.059 | 0.077 | -0.77 | 0.440 | s(day):treatmentTL | 1.000 | 1.000 | 0.000 | 0.992 |
| N:L | 0.028 | 1 | 5.53 | 0.021 * | 0.147 | 0.078 | 1.89 | 0.060 | s(day):treatmentNL | 2.250 | 2.791 | 12.594 | <0.001 *** |
| C:L | 0.037 | 1 | 7.23 | 0.009 ** | -0.225 | 0.098 | -2.29 | 0.022 * | s(day):treatmentCL | 1.000 | 1.000 | 15.394 | <0.001 *** |
| T:N:C | 0.005 | 1 | 1.06 | 0.306 | 0.017 | 0.104 | 0.16 | 0.870 | s(day):treatmentTNC | 1.000 | 1.000 | 1.154 | 0.283 |
| T:N:L | 0.027 | 1 | 5.21 | 0.025 * | -0.083 | 0.107 | -0.77 | 0.440 | s(day):treatmentTNL | 1.810 | 2.250 | 4.723 | 0.008 |
| T:C:L | 0.013 | 1 | 2.47 | 0.120 | 0.281 | 0.119 | 2.35 | 0.019 * | s(day):treatmentTCL | 1.000 | 1.000 | 23.786 | <0.001 *** |
| N:C:L | 0.024 | 1 | 4.60 | 0.035 * | -0.038 | 0.121 | -0.32 | 0.751 | s(day):treatmentNCL | 2.006 | 2.493 | 4.437 | 0.010 ** |
| T:N:C:L | 0.006 | 1 | 1.23 | 0.270 | -0.147 | 0.158 | -0.93 | 0.353 | s(day):treatmentTNCL | 1.586 | 1.961 | 12.040 | <0.001 *** |
| Residuals | 0.411 | 80 | | | | | | | | | | | |

## Dynamics of *Stentor coeruleus*

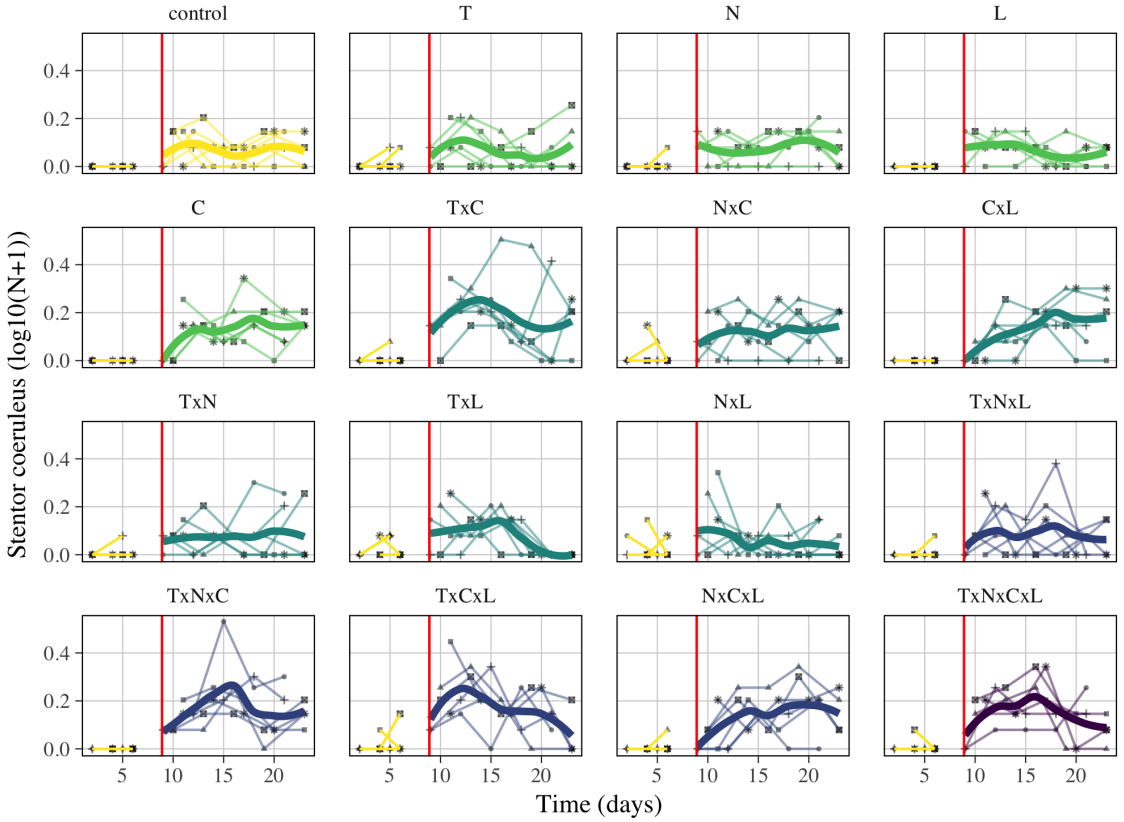



*Figure S20: Time serie of the abundance of Stentor coeruleus. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S18: Statisitcal analysis of Stentor coeruleus's densities. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e., **Pre-perturbation**). The time series were analysed with a GAM explaining 29.3% of deviance (Adj. $R^2$ = 22.7%), with the main effects and their interactions as **parametric coefficients** and the temporal change with a spline function the for each treatment (i.e., **smooth term**).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 0.000 | 1 | 0.00 | 1.000 | 0.003 | 0.016 | 0.22 | 0.828 | s(day):treatmentno.change | 1.000 | 1.000 | 0.000 | 0.994 |
| Temperature (T) | 0.002 | 1 | 2.32 | 0.132 | -0.003 | 0.022 | -0.11 | 0.909 | s(day):treatmentT | 1.000 | 1.000 | 0.148 | 0.701 |
| Nutrient (N) | 0.001 | 1 | 0.58 | 0.449 | 0.005 | 0.022 | 0.22 | 0.828 | s(day):treatmentN | 1.000 | 1.000 | 0.155 | 0.694 |
| Carbon (C) | 0.000 | 1 | 0.00 | 1.000 | 0.054 | 0.022 | 2.40 | 0.017 * | s(day):treatmentC | 1.713 | 2.128 | 1.971 | 0.128 |
| Light (L) | 0.000 | 1 | 0.00 | 1.000 | -0.006 | 0.022 | -0.26 | 0.795 | s(day):treatmentL | 1.000 | 1.000 | 0.716 | 0.398 |
| T:N | 0.001 | 1 | 1.16 | 0.285 | 0.003 | 0.032 | 0.09 | 0.932 | s(day):treatmentTN | 1.000 | 1.000 | 0.411 | 0.522 |
| T:C | 0.000 | 1 | 0.29 | 0.592 | 0.059 | 0.032 | 1.87 | 0.063 | s(day):treatmentTC | 2.307 | 2.863 | 0.968 | 0.311 |
| N:C | 0.000 | 1 | 0.00 | 1.000 | -0.013 | 0.032 | -0.40 | 0.692 | s(day):treatmentNC | 1.000 | 1.000 | 0.895 | 0.345 |
| T:L | 0.000 | 1 | 0.29 | 0.592 | 0.010 | 0.032 | 0.31 | 0.754 | s(day):treatmentTL | 1.997 | 2.483 | 3.093 | 0.033 * |
| N:L | 0.000 | 1 | 0.00 | 1.000 | -0.009 | 0.032 | -0.28 | 0.780 | s(day):treatmentNL | 1.000 | 1.000 | 1.801 | 0.180 |
| C:L | 0.000 | 1 | 0.00 | 1.000 | 0.011 | 0.032 | 0.35 | 0.729 | s(day):treatmentCL | 1.711 | 2.127 | 5.250 | 0.005 ** |
| T:N:C | 0.000 | 1 | 0.00 | 1.000 | -0.011 | 0.045 | -0.24 | 0.809 | s(day):treatmentTNC | 3.513 | 4.321 | 2.286 | 0.047 * |
| T:N:L | 0.000 | 1 | 0.14 | 0.705 | 0.010 | 0.045 | 0.22 | 0.828 | s(day):treatmentTNL | 1.162 | 1.305 | 0.028 | 0.891 |
| T:C:L | 0.000 | 1 | 0.00 | 0.953 | -0.030 | 0.045 | -0.68 | 0.499 | s(day):treatmentTCL | 2.022 | 2.513 | 3.687 | 0.016 * |
| N:C:L | 0.000 | 1 | 0.00 | 1.000 | 0.020 | 0.045 | 0.44 | 0.658 | s(day):treatmentNCL | 1.890 | 2.350 | 3.668 | 0.022 * |
| T:N:C:L | 0.000 | 1 | 0.00 | 0.967 | -0.027 | 0.064 | -0.42 | 0.673 | s(day):treatmentTNCL | 2.580 | 3.195 | 2.234 | 0.075 |
| Residuals | 0.072 | 80 | | | | | | | | | | | |

## Dynamics of *Dileptus anser*

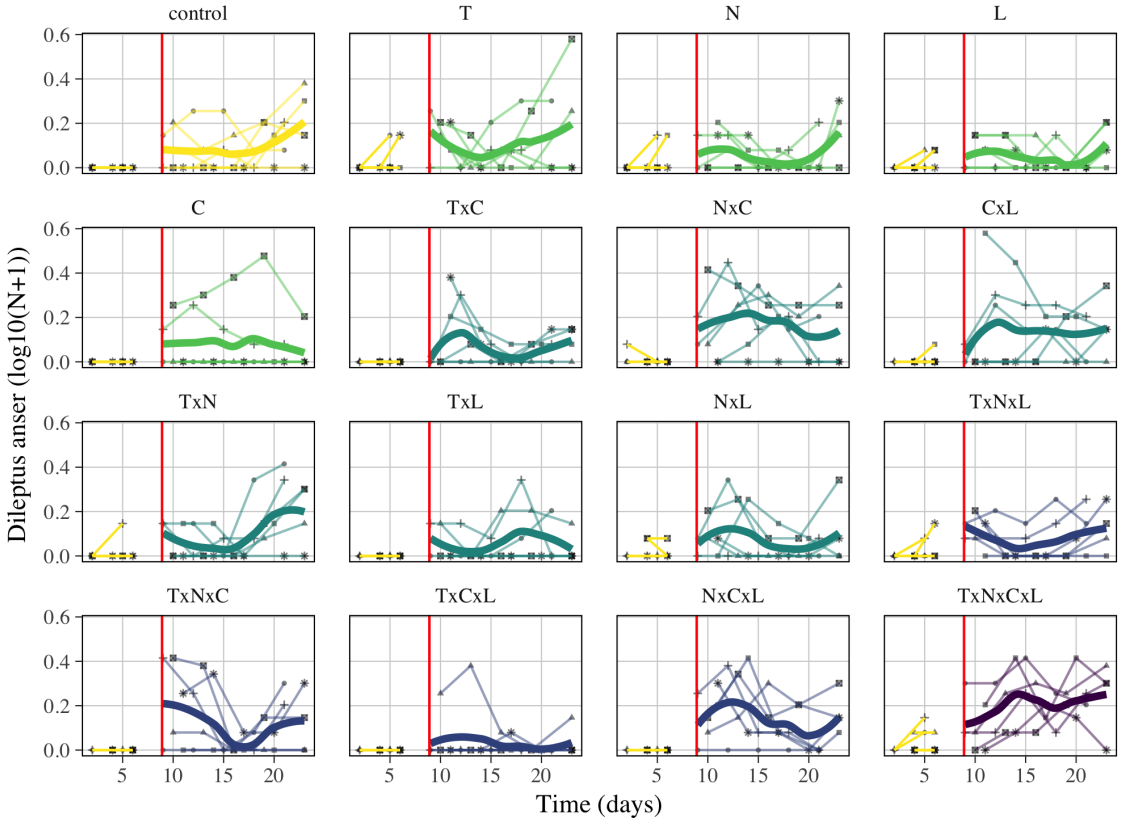



*Figure S21: Time serie of the abundance of Dileptus anser. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S19: Statisitcal analysis of Dileptus anser's densities. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 25.7% of deviance (Adj. $R^2$ = 19.4%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****). Despite some differences between treatments in pre-perturbation, we did not add the mean pre-perturbation as random effect in the GAM, before application some Dileptus anser were added in the microcosms.*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 0.000 | 1 | 0.00 | 1.000 | 0.001 | 0.021 | 0.03 | 0.974 | s(day):treatmentno.change | 1.000 | 1.000 | 0.004 | 0.948 |
| Temperature (T) | 0.007 | 1 | 4.31 | 0.041 * | 0.012 | 0.029 | 0.40 | 0.687 | s(day):treatmentT | 1.000 | 1.000 | 0.348 | 0.555 |
| Nutrient (N) | 0.007 | 1 | 4.31 | 0.041 * | -0.035 | 0.029 | -1.20 | 0.232 | s(day):treatmentN | 1.000 | 1.000 | 1.344 | 0.247 |
| Carbon (C) | 0.000 | 1 | 0.00 | 1.000 | -0.023 | 0.029 | -0.78 | 0.437 | s(day):treatmentC | 1.949 | 2.423 | 3.219 | 0.038 * |
| Light (L) | 0.005 | 1 | 2.85 | 0.096 | -0.048 | 0.029 | -1.63 | 0.105 | s(day):treatmentL | 1.000 | 1.000 | 3.134 | 0.077 |
| T:N | 0.008 | 1 | 4.85 | 0.031 * | 0.026 | 0.042 | 0.64 | 0.526 | s(day):treatmentTN | 1.000 | 1.000 | 0.733 | 0.392 |
| T:C | 0.004 | 1 | 2.15 | 0.146 | -0.019 | 0.042 | -0.47 | 0.642 | s(day):treatmentTC | 1.000 | 1.000 | 3.866 | 0.050 * |
| N:C | 0.004 | 1 | 2.15 | 0.146 | 0.123 | 0.042 | 2.97 | 0.003 ** | s(day):treatmentNC | 2.091 | 2.598 | 3.953 | 0.011 * |
| T:L | 0.012 | 1 | 7.08 | 0.009 ** | -0.008 | 0.042 | -0.19 | 0.851 | s(day):treatmentTL | 2.261 | 2.806 | 2.190 | 0.145 |
| N:L | 0.008 | 1 | 5.12 | 0.026 * | 0.058 | 0.042 | 1.40 | 0.162 | s(day):treatmentNL | 1.000 | 1.000 | 4.814 | 0.029 * |
| C:L | 0.001 | 1 | 0.63 | 0.429 | 0.106 | 0.042 | 2.55 | 0.011 * | s(day):treatmentCL | 2.106 | 2.616 | 1.652 | 0.145 |
| T:N:C | 0.004 | 1 | 2.42 | 0.124 | -0.065 | 0.059 | -1.12 | 0.265 | s(day):treatmentTNC | 1.000 | 1.000 | 9.269 | 0.002 ** |
| T:N:L | 0.014 | 1 | 8.52 | 0.005 ** | -0.022 | 0.059 | -0.38 | 0.708 | s(day):treatmentTNL | 1.000 | 1.000 | 1.915 | 0.167 |
| T:C:L | 0.004 | 1 | 2.56 | 0.114 | -0.089 | 0.059 | -1.52 | 0.130 | s(day):treatmentTCL | 1.277 | 1.501 | 2.994 | 0.045 * |
| N:C:L | 0.003 | 1 | 1.74 | 0.191 | -0.142 | 0.059 | -2.42 | 0.016 * | s(day):treatmentNCL | 1.879 | 2.337 | 4.434 | 0.008 ** |
| T:N:C:L | 0.002 | 1 | 1.21 | 0.274 | 0.228 | 0.083 | 2.75 | 0.006 ** | s(day):treatmentTNCL | 2.076 | 2.580 | 1.318 | 0.260 |
| Residuals | 0.132 | 80 | | | | | | | | | | | |

## Simpson's indice

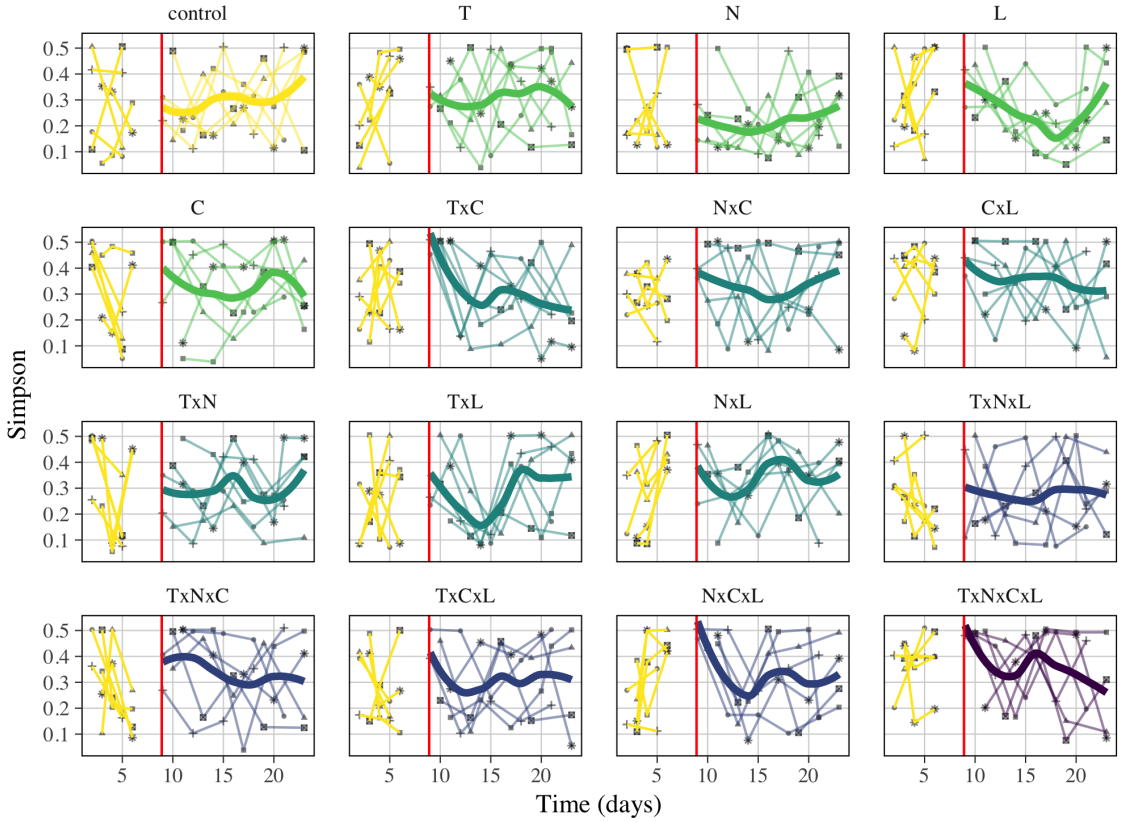



*Figure S22: Time serie of the Simpson's indice. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S20: Statisitcal analysis of the Simpson's indice over time. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 17.7% of deviance (Adj. $R^2$ = 11.2%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 0.410 | 1 | 19.35 | <0.001 *** | -0.001 | 0.009 | -0.15 | 0.882 | s(day):treatmentno.change | 1.000 | 1.000 | 0.005 | 0.943 |
| Temperature (T) | 0.028 | 1 | 1.31 | 0.256 | 0.004 | 0.012 | 0.31 | 0.754 | s(day):treatmentT | 1.000 | 1.000 | 0.807 | 0.369 |
| Nutrient (N) | 0.002 | 1 | 0.12 | 0.733 | -0.027 | 0.012 | -2.26 | 0.024 * | s(day):treatmentN | 1.000 | 1.000 | 0.177 | 0.674 |
| Carbon (C) | 0.003 | 1 | 0.16 | 0.688 | 0.011 | 0.012 | 0.95 | 0.343 | s(day):treatmentC | 1.000 | 1.000 | 1.476 | 0.225 |
| Light (L) | 0.021 | 1 | 1.01 | 0.317 | -0.010 | 0.012 | -0.82 | 0.413 | s(day):treatmentL | 2.125 | 2.648 | 2.849 | 0.053 |
| T:N | 0.025 | 1 | 1.19 | 0.279 | 0.022 | 0.017 | 1.32 | 0.187 | s(day):treatmentTN | 1.000 | 1.000 | 0.536 | 0.465 |
| T:C | 0.000 | 1 | 0.00 | 0.947 | -0.013 | 0.017 | -0.77 | 0.443 | s(day):treatmentTC | 1.003 | 1.007 | 12.532 | <0.001 *** |
| N:C | 0.001 | 1 | 0.03 | 0.871 | 0.028 | 0.017 | 1.62 | 0.106 | s(day):treatmentNC | 1.060 | 1.118 | 1.084 | 0.325 |
| T:L | 0.032 | 1 | 1.49 | 0.225 | 0.001 | 0.017 | 0.05 | 0.958 | s(day):treatmentTL | 3.454 | 4.258 | 1.014 | 0.357 |
| N:L | 0.007 | 1 | 0.35 | 0.558 | 0.050 | 0.017 | 2.92 | 0.004 ** | s(day):treatmentNL | 1.000 | 1.000 | 0.681 | 0.410 |
| C:L | 0.005 | 1 | 0.25 | 0.618 | 0.015 | 0.017 | 0.89 | 0.377 | s(day):treatmentCL | 1.000 | 1.000 | 5.234 | 0.023 * |
| T:N:C | 0.005 | 1 | 0.22 | 0.640 | -0.012 | 0.024 | -0.51 | 0.609 | s(day):treatmentTNC | 1.000 | 1.000 | 5.925 | 0.015 * |
| T:N:L | 0.001 | 1 | 0.06 | 0.801 | -0.047 | 0.024 | -1.94 | 0.054 | s(day):treatmentTNL | 1.000 | 1.000 | 1.325 | 0.250 |
| T:C:L | 0.007 | 1 | 0.31 | 0.578 | -0.004 | 0.024 | -0.19 | 0.852 | s(day):treatmentTCL | 1.000 | 1.000 | 1.372 | 0.242 |
| N:C:L | 0.010 | 1 | 0.47 | 0.495 | -0.058 | 0.024 | -2.39 | 0.017 * | s(day):treatmentNCL | 2.164 | 2.692 | 2.767 | 0.036 * |
| T:N:C:L | 0.063 | 1 | 2.98 | 0.088 | 0.059 | 0.034 | 1.73 | 0.084 | s(day):treatmentTNCL | 1.000 | 1.000 | 9.943 | 0.002 ** |
| Residuals | 1.697 | 80 | | | | | | | | | | | |

## Richness

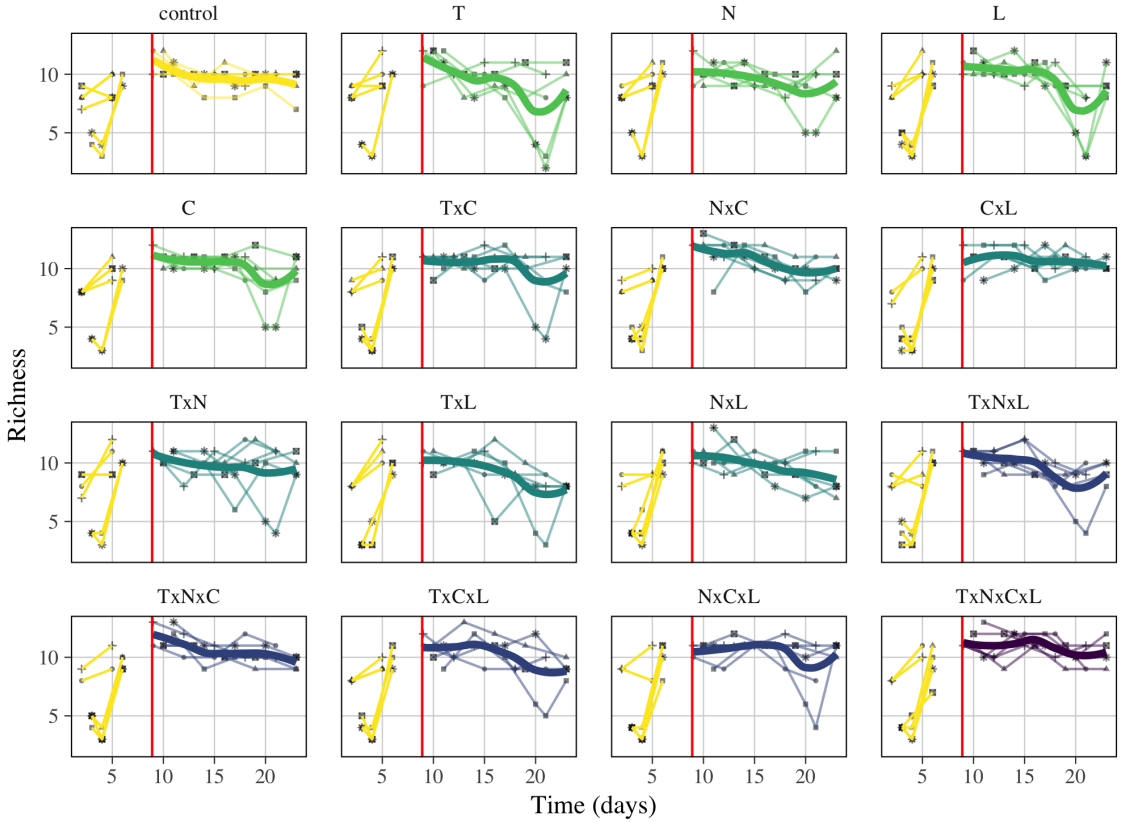



*Figure S23: Time serie of the Richness. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S21: Statisitcal analysis of the Richness over time. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 40.8% of deviance (Adj.* $R^2$ *= 32.9%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 504.170 | 1 | 479.21 | <0.001 *** | 0.001 | 0.013 | 0.09 | 0.926 | s(day):treatmentno.change | 1.000 | 1.000 | 0.007 | 0.935 |
| Temperature (T) | 2.080 | 1 | 1.98 | 0.163 | -0.043 | 0.018 | -2.44 | 0.015 * | s(day):treatmentT | 6.602 | 7.687 | 8.752 | <0.001 *** |
| Nutrient (N) | 2.080 | 1 | 1.98 | 0.163 | -0.020 | 0.018 | -1.14 | 0.256 | s(day):treatmentN | 1.000 | 1.000 | 0.308 | 0.579 |
| Carbon (C) | 1.330 | 1 | 1.27 | 0.264 | 0.006 | 0.018 | 0.35 | 0.726 | s(day):treatmentC | 4.267 | 5.214 | 2.178 | 0.063 |
| Light (L) | 4.080 | 1 | 3.88 | 0.052 | -0.030 | 0.018 | -1.70 | 0.090 | s(day):treatmentL | 6.099 | 7.206 | 8.120 | <0.001 *** |
| T:N | 0.670 | 1 | 0.63 | 0.428 | 0.054 | 0.025 | 2.16 | 0.032 * | s(day):treatmentTN | 1.000 | 1.000 | 0.042 | 0.837 |
| T:C | 0.380 | 1 | 0.36 | 0.552 | 0.043 | 0.025 | 1.72 | 0.086 | s(day):treatmentTC | 4.248 | 5.192 | 2.123 | 0.071 |
| N:C | 1.040 | 1 | 0.99 | 0.323 | 0.045 | 0.025 | 1.80 | 0.072 | s(day):treatmentNC | 1.000 | 1.000 | 0.223 | 0.637 |
| T:L | 1.040 | 1 | 0.99 | 0.323 | 0.030 | 0.025 | 1.20 | 0.231 | s(day):treatmentTL | 3.789 | 4.655 | 3.443 | 0.008 ** |
| N:L | 2.670 | 1 | 2.53 | 0.115 | 0.046 | 0.025 | 1.82 | 0.069 | s(day):treatmentNL | 1.000 | 1.000 | 0.575 | 0.449 |
| C:L | 1.040 | 1 | 0.99 | 0.323 | 0.059 | 0.025 | 2.37 | 0.018 * | s(day):treatmentCL | 1.000 | 1.000 | 0.995 | 0.319 |
| T:N:C | 0.020 | 1 | 0.02 | 0.888 | -0.054 | 0.035 | -1.52 | 0.129 | s(day):treatmentTNC | 1.000 | 1.000 | 0.097 | 0.756 |
| T:N:L | 0.330 | 1 | 0.32 | 0.575 | -0.053 | 0.035 | -1.51 | 0.132 | s(day):treatmentTNL | 3.492 | 4.303 | 2.265 | 0.063 |
| T:C:L | 0.000 | 1 | 0.00 | 1.000 | -0.060 | 0.035 | -1.69 | 0.093 | s(day):treatmentTCL | 2.134 | 2.656 | 1.175 | 0.240 |
| N:C:L | 0.750 | 1 | 0.71 | 0.401 | -0.091 | 0.036 | -2.56 | 0.011 * | s(day):treatmentNCL | 4.444 | 5.419 | 2.419 | 0.041 * |
| T:N:C:L | 0.010 | 1 | 0.01 | 0.921 | 0.108 | 0.050 | 2.15 | 0.032 * | s(day):treatmentTNCL | 1.000 | 1.000 | 0.374 | 0.542 |
| Residuals | 84.170 | 80 | | | | | | | | | | | |

## Connectance

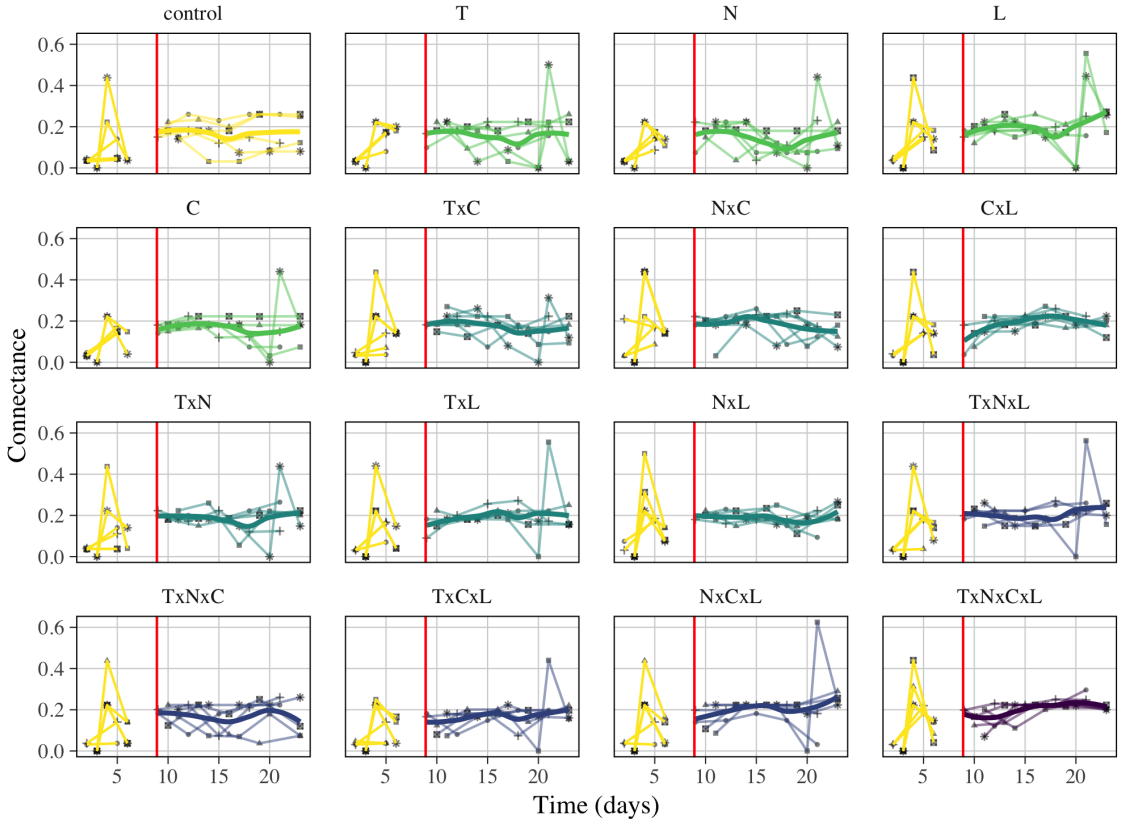



*Figure S24: Time serie of the Connectance. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S22: Statisitcal analysis of the Connectance over time. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e., **Pre-perturbation**). The time series were analysed with a GAM explaining 12.9% of deviance (Adj. $R^2$ = 6%), with the main effects and their interactions as **parametric coefficients** and the temporal change with a spline function the for each treatment (i.e., **smooth term**). Due to differences between treatments in pre-perturbation, we added the mean pre-perturbation as random effect in the GAM.*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 0.021 | 1 | 8.04 | 0.006 ** | 0.000 | 0.005 | 0.02 | 0.981 | s(day):treatmentno.change | 1.000 | 1.000 | 0.054 | 0.817 |
| Temperature (T) | 0.032 | 1 | 12.62 | <0.001 *** | -0.004 | 0.007 | -0.55 | 0.585 | s(day):treatmentT | 1.000 | 1.000 | 0.077 | 0.782 |
| Nutrient (N) | 0.016 | 1 | 6.22 | 0.015 * | -0.008 | 0.007 | -1.18 | 0.240 | s(day):treatmentN | 1.000 | 1.001 | 0.197 | 0.658 |
| Carbon (C) | 0.016 | 1 | 6.34 | 0.014 * | -0.001 | 0.007 | -0.20 | 0.840 | s(day):treatmentC | 1.000 | 1.000 | 0.219 | 0.640 |
| Light (L) | 0.023 | 1 | 9.11 | 0.003 ** | 0.012 | 0.007 | 1.70 | 0.091 | s(day):treatmentL | 1.000 | 1.000 | 2.679 | 0.102 |
| T:N | 0.034 | 1 | 13.32 | <0.001 *** | 0.019 | 0.010 | 1.98 | 0.048 * | s(day):treatmentTN | 1.000 | 1.000 | 0.087 | 0.768 |
| T:C | 0.023 | 1 | 8.82 | 0.004 ** | 0.006 | 0.010 | 0.67 | 0.504 | s(day):treatmentTC | 1.000 | 1.000 | 0.936 | 0.334 |
| N:C | 0.006 | 1 | 2.21 | 0.141 | 0.014 | 0.010 | 1.40 | 0.161 | s(day):treatmentNC | 1.682 | 2.089 | 0.873 | 0.414 |
| T:L | 0.032 | 1 | 12.37 | <0.001 *** | 0.001 | 0.010 | 0.12 | 0.905 | s(day):treatmentTL | 1.505 | 1.849 | 1.219 | 0.381 |
| N:L | 0.011 | 1 | 4.45 | 0.038 * | 0.004 | 0.010 | 0.44 | 0.664 | s(day):treatmentNL | 1.000 | 1.000 | 0.008 | 0.927 |
| C:L | 0.012 | 1 | 4.77 | 0.032 * | -0.003 | 0.010 | -0.34 | 0.735 | s(day):treatmentCL | 2.214 | 2.749 | 2.589 | 0.054 |
| T:N:C | 0.010 | 1 | 3.87 | 0.053 | -0.022 | 0.014 | -1.60 | 0.109 | s(day):treatmentTNC | 1.000 | 1.000 | 0.034 | 0.854 |
| T:N:L | 0.028 | 1 | 10.81 | 0.002 ** | -0.010 | 0.014 | -0.76 | 0.450 | s(day):treatmentTNL | 1.000 | 1.000 | 0.918 | 0.339 |
| T:C:L | 0.012 | 1 | 4.73 | 0.033 * | -0.012 | 0.014 | -0.84 | 0.401 | s(day):treatmentTCL | 1.000 | 1.000 | 2.372 | 0.124 |
| N:C:L | 0.001 | 1 | 0.41 | 0.525 | -0.006 | 0.014 | -0.44 | 0.662 | s(day):treatmentNCL | 1.000 | 1.000 | 3.117 | 0.078 |
| T:N:C:L | 0.002 | 1 | 0.82 | 0.368 | 0.023 | 0.020 | 1.15 | 0.249 | s(day):treatmentTNCL | 1.415 | 1.718 | 3.027 | 0.098 |
| Residuals | 0.204 | 80 | | | | | | | | | | | |

## Community respiration

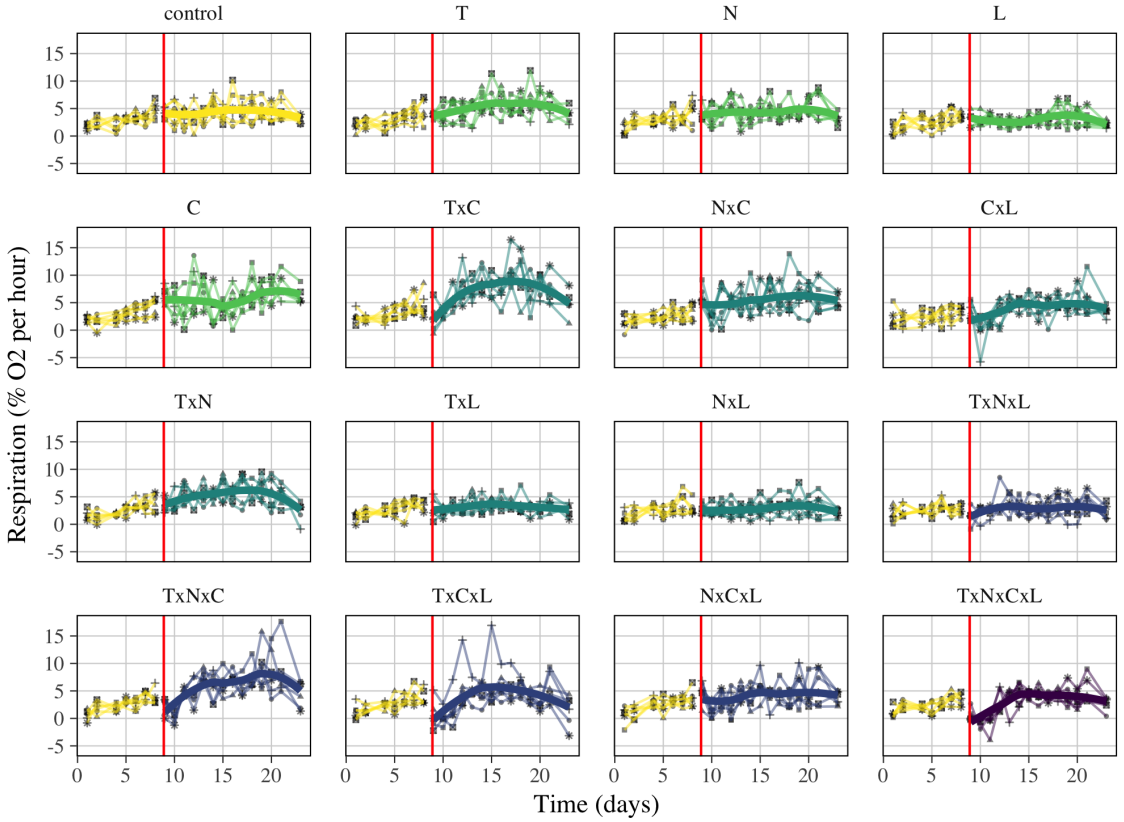



*Figure S25: Time serie of the respiration (% of dissolved oxygen consumed per hour). For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S23: Statisitcal analysis of the respiration over time. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 39.5% of deviance (Adj. $R^2$ = 36.7%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 81.205 | 1 | 112.51 | <0.001 *** | -0.001 | 0.009 | -0.11 | 0.914 | s(day):treatmentno.change | 1.000 | 1.000 | 0.03 | 0.872 |
| Temperature (T) | 0.281 | 1 | 0.39 | 0.535 | 0.035 | 0.013 | 2.77 | 0.006 ** | s(day):treatmentT | 1.381 | 1.669 | 1.34 | 0.179 |
| Nutrient (N) | 0.005 | 1 | 0.01 | 0.936 | 0.005 | 0.013 | 0.43 | 0.665 | s(day):treatmentN | 1.000 | 1.000 | 0.35 | 0.553 |
| Carbon (C) | 0.128 | 1 | 0.18 | 0.675 | 0.052 | 0.013 | 4.17 | <0.001 *** | s(day):treatmentC | 3.994 | 4.914 | 3.79 | 0.002 ** |
| Light (L) | 0.569 | 1 | 0.79 | 0.377 | -0.048 | 0.013 | -3.82 | <0.001 *** | s(day):treatmentL | 1.001 | 1.001 | 0.13 | 0.725 |
| T:N | 0.152 | 1 | 0.21 | 0.647 | -0.007 | 0.018 | -0.38 | 0.704 | s(day):treatmentTN | 2.573 | 3.209 | 2.36 | 0.069 |
| T:C | 0.003 | 1 | 0.00 | 0.952 | 0.002 | 0.018 | 0.09 | 0.932 | s(day):treatmentTC | 3.958 | 4.871 | 11.18 | <0.001 *** |
| N:C | 1.187 | 1 | 1.65 | 0.203 | -0.016 | 0.018 | -0.93 | 0.353 | s(day):treatmentNC | 1.000 | 1.000 | 4.80 | 0.029 * |
| T:L | 0.003 | 1 | 0.00 | 0.951 | -0.030 | 0.018 | -1.68 | 0.093 | s(day):treatmentTL | 1.000 | 1.000 | 0.02 | 0.876 |
| N:L | 0.001 | 1 | 0.00 | 0.966 | -0.015 | 0.018 | -0.87 | 0.386 | s(day):treatmentNL | 1.000 | 1.000 | 0.32 | 0.569 |
| C:L | 0.232 | 1 | 0.32 | 0.572 | -0.027 | 0.018 | -1.51 | 0.132 | s(day):treatmentCL | 7.688 | 8.550 | 7.44 | <0.001 *** |
| T:N:C | 0.211 | 1 | 0.29 | 0.590 | -0.019 | 0.025 | -0.74 | 0.459 | s(day):treatmentTNC | 4.750 | 5.784 | 11.39 | <0.001 *** |
| T:N:L | 0.041 | 1 | 0.06 | 0.812 | 0.001 | 0.025 | 0.02 | 0.982 | s(day):treatmentTNL | 1.000 | 1.000 | 1.44 | 0.230 |
| T:C:L | 0.152 | 1 | 0.21 | 0.647 | -0.014 | 0.025 | -0.55 | 0.580 | s(day):treatmentTCL | 4.521 | 5.523 | 12.73 | <0.001 *** |
| N:C:L | 0.927 | 1 | 1.28 | 0.261 | 0.038 | 0.025 | 1.53 | 0.128 | s(day):treatmentNCL | 1.000 | 1.000 | 6.32 | 0.012 * |
| T:N:C:L | 0.142 | 1 | 0.20 | 0.658 | -0.017 | 0.035 | -0.47 | 0.641 | s(day):treatmentTNCL | 5.642 | 6.760 | 11.71 | <0.001 *** |
| Residuals | 57.739 | 80 | | | | | | | | | | | |

## Net Primary Production

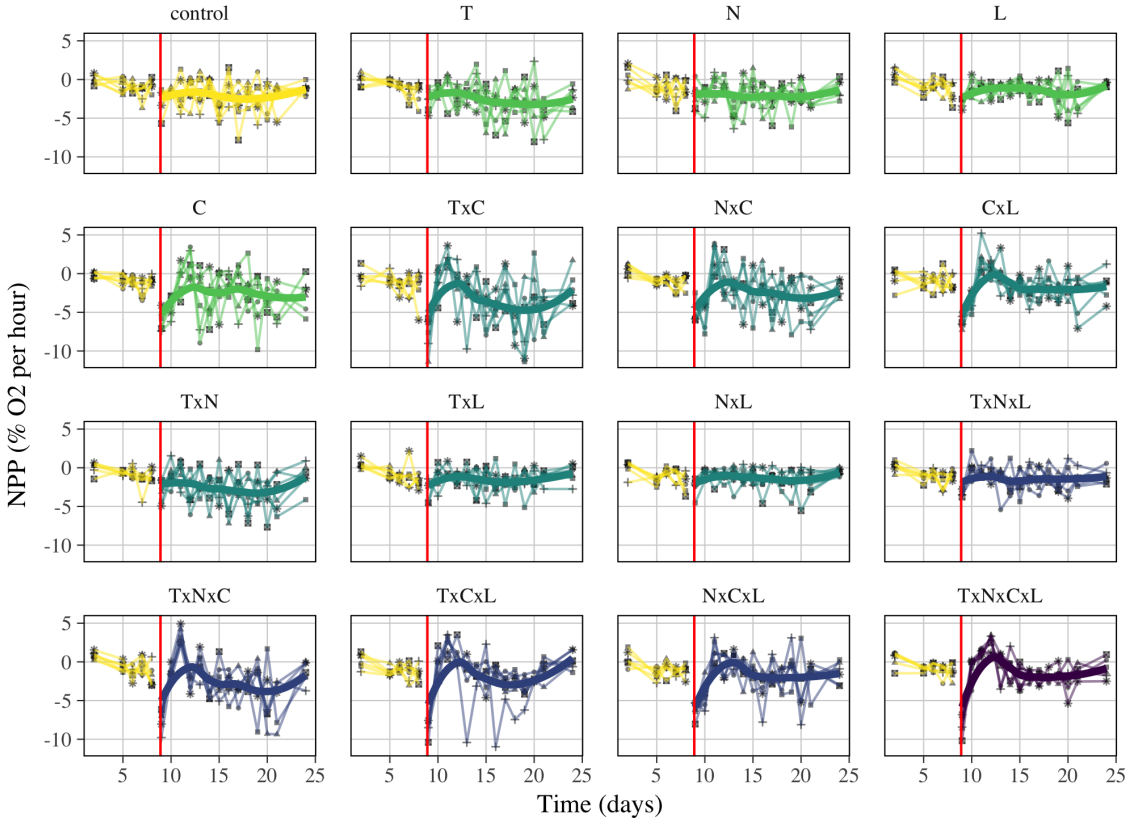



*Figure S26: Time serie of the Net Primary Production (NPP). For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S24: Statisitcal analysis of the net primary production over time. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e.,* ***Pre-perturbation****). The time series were analysed with a GAM explaining 36.1% of deviance (Adj. $R^2$ = 32.3%), with the main effects and their interactions as* ***parametric coefficients*** *and the temporal change with a spline function the for each treatment (i.e.,* ***smooth term****).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 7.171 | 1 | 38.98 | <0.001 *** | 0.002 | 0.026 | 0.06 | 0.952 | s(day):treatmentno.change | 1.000 | 1.000 | 0.001 | 0.978 |
| Temperature (T) | 0.278 | 1 | 1.51 | 0.222 | -0.057 | 0.037 | -1.55 | 0.121 | s(day):treatmentT | 1.000 | 1.000 | 4.024 | 0.045 * |
| Nutrient (N) | 0.099 | 1 | 0.54 | 0.465 | 0.008 | 0.037 | 0.21 | 0.834 | s(day):treatmentN | 1.000 | 1.000 | 0.005 | 0.943 |
| Carbon (C) | 0.046 | 1 | 0.25 | 0.619 | -0.087 | 0.037 | -2.38 | 0.018 | s(day):treatmentC | 6.696 | 7.769 | 4.472 | <0.001 *** |
| Light (L) | 0.066 | 1 | 0.36 | 0.552 | 0.063 | 0.037 | 1.72 | 0.086 | s(day):treatmentL | 1.313 | 1.563 | 0.141 | 0.824 |
| T:N | 0.324 | 1 | 1.76 | 0.188 | -0.005 | 0.052 | -0.09 | 0.927 | s(day):treatmentTN | 1.025 | 1.050 | 0.762 | 0.372 |
| T:C | 0.007 | 1 | 0.04 | 0.851 | -0.080 | 0.052 | -1.56 | 0.120 | s(day):treatmentTC | 8.353 | 8.877 | 15.216 | <0.001 *** |
| N:C | 0.197 | 1 | 1.07 | 0.304 | 0.018 | 0.052 | 0.35 | 0.725 | s(day):treatmentNC | 3.702 | 4.540 | 2.400 | 0.053 |
| T:L | 0.017 | 1 | 0.09 | 0.764 | 0.054 | 0.052 | 1.04 | 0.297 | s(day):treatmentTL | 1.000 | 1.000 | 0.195 | 0.659 |
| N:L | 0.000 | 1 | 0.00 | 0.973 | 0.007 | 0.052 | 0.14 | 0.885 | s(day):treatmentNL | 1.000 | 1.000 | 0.011 | 0.915 |
| C:L | 0.002 | 1 | 0.01 | 0.914 | 0.044 | 0.052 | 0.85 | 0.395 | s(day):treatmentCL | 5.389 | 6.458 | 4.986 | <0.001 *** |
| T:N:C | 0.032 | 1 | 0.17 | 0.680 | 0.106 | 0.073 | 1.45 | 0.146 | s(day):treatmentTNC | 7.316 | 8.283 | 9.661 | <0.001 *** |
| T:N:L | 0.016 | 1 | 0.09 | 0.771 | 0.001 | 0.073 | 0.02 | 0.984 | s(day):treatmentTNL | 1.000 | 1.000 | 0.005 | 0.942 |
| T:C:L | 0.081 | 1 | 0.44 | 0.509 | 0.031 | 0.073 | 0.42 | 0.672 | s(day):treatmentTCL | 7.847 | 8.637 | 12.157 | <0.001 *** |
| N:C:L | 0.004 | 1 | 0.02 | 0.891 | -0.048 | 0.073 | -0.66 | 0.513 | s(day):treatmentNCL | 5.101 | 6.144 | 5.868 | <0.001 *** |
| T:N:C:L | 0.001 | 1 | 0.01 | 0.938 | -0.020 | 0.103 | -0.19 | 0.849 | s(day):treatmentTNCL | 7.047 | 8.071 | 11.330 | <0.001 *** |
| Residuals | 14.719 | 80 | | | | | | | | | | | |

## Total biomass

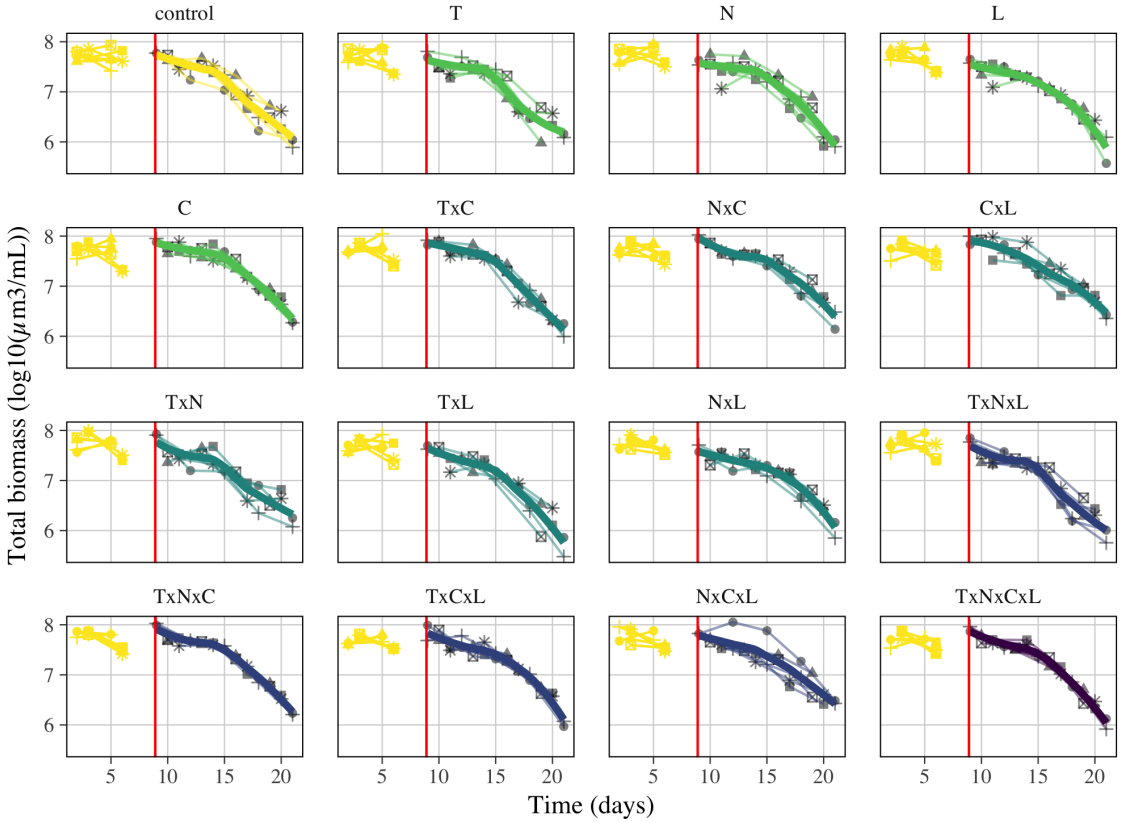



*Figure S27: Time serie of the total biomass. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances). A loess regression fit the time serie of the six replicates from the day of treatment application (thick line), and the symbols indicates the replicate.*

*Table S25: Statisitcal analysis of the total biomass over time. We checked the presence of difference between the treatments (ANOVA type III) before the application of the disturbances (i.e., **Pre-perturbation**). The time series were analysed with a GAM explaining 51.7% of deviance (Adj. $R^2$ = 45.7%), with the main effects and their interactions as **parametric coefficients** and the temporal change with a spline function the for each treatment (i.e., **smooth term**).*

| | **Pre-perturbation** | | | | **Parametric coefficient** | | | | **Smooth term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sum Sq | Df | F | Pr(>F) | Estimate | Std. Error | t | Pr(>\|t\|) | | edf | Ref.df | F | p-value |
| (Intercept) | 353.740 | 1 | 10378.71 | <0.001 *** | -0.003 | 0.032 | -0.08 | 0.937 | s(day):treatmentno.change | 1.000 | 1.000 | 0.034 | 0.855 |
| Temperature (T) | 0.000 | 1 | 0.07 | 0.795 | -0.026 | 0.045 | -0.57 | 0.570 | s(day):treatmentT | 1.000 | 1.000 | 1.299 | 0.255 |
| Nutrient (N) | 0.010 | 1 | 0.27 | 0.603 | -0.013 | 0.045 | -0.30 | 0.768 | s(day):treatmentN | 2.570 | 3.197 | 2.502 | 0.058 |
| Carbon (C) | 0.010 | 1 | 0.39 | 0.535 | 0.277 | 0.045 | 6.16 | <0.001 *** | s(day):treatmentC | 1.753 | 2.184 | 3.751 | 0.025 * |
| Light (L) | 0.050 | 1 | 1.40 | 0.240 | -0.068 | 0.045 | -1.52 | 0.129 | s(day):treatmentL | 3.061 | 3.800 | 2.924 | 0.033 * |
| T:N | 0.000 | 1 | 0.08 | 0.782 | 0.086 | 0.064 | 1.36 | 0.176 | s(day):treatmentTN | 1.479 | 1.812 | 3.041 | 0.037 * |
| T:C | 0.010 | 1 | 0.21 | 0.647 | -0.082 | 0.064 | -1.29 | 0.197 | s(day):treatmentTC | 1.710 | 2.127 | 0.863 | 0.448 |
| N:C | 0.000 | 1 | 0.04 | 0.849 | 0.008 | 0.064 | 0.13 | 0.897 | s(day):treatmentNC | 2.558 | 3.182 | 3.360 | 0.017 * |
| T:L | 0.020 | 1 | 0.47 | 0.496 | -0.025 | 0.064 | -0.39 | 0.699 | s(day):treatmentTL | 2.066 | 2.576 | 1.276 | 0.400 |
| N:L | 0.000 | 1 | 0.01 | 0.926 | 0.089 | 0.064 | 1.40 | 0.164 | s(day):treatmentNL | 2.999 | 3.724 | 4.817 | 0.002 ** |
| C:L | 0.020 | 1 | 0.57 | 0.452 | 0.067 | 0.064 | 1.06 | 0.290 | s(day):treatmentCL | 1.304 | 1.545 | 4.293 | 0.016 * |
| T:N:C | 0.000 | 1 | 0.04 | 0.851 | -0.026 | 0.090 | -0.29 | 0.770 | s(day):treatmentTNC | 1.201 | 1.374 | 1.963 | 0.191 |
| T:N:L | 0.000 | 1 | 0.01 | 0.933 | -0.117 | 0.090 | -1.30 | 0.195 | s(day):treatmentTNL | 1.000 | 1.000 | 0.000 | 0.988 |
| T:C:L | 0.010 | 1 | 0.26 | 0.614 | 0.015 | 0.090 | 0.16 | 0.870 | s(day):treatmentTCL | 3.238 | 4.017 | 2.559 | 0.038 * |
| N:C:L | 0.000 | 1 | 0.02 | 0.892 | -0.170 | 0.090 | -1.89 | 0.060 | s(day):treatmentNCL | 1.000 | 1.000 | 11.991 | <0.001 *** |
| T:N:C:L | 0.000 | 1 | 0.008 | 0.929 | 0.115 | 0.127 | 0.90 | 0.369 | s(day):treatmentTNCL | 2.365 | 2.944 | 1.278 | 0.356 |
| Residuals | 2.730 | 80 | | | | | | | | | | | |

## Decomposition

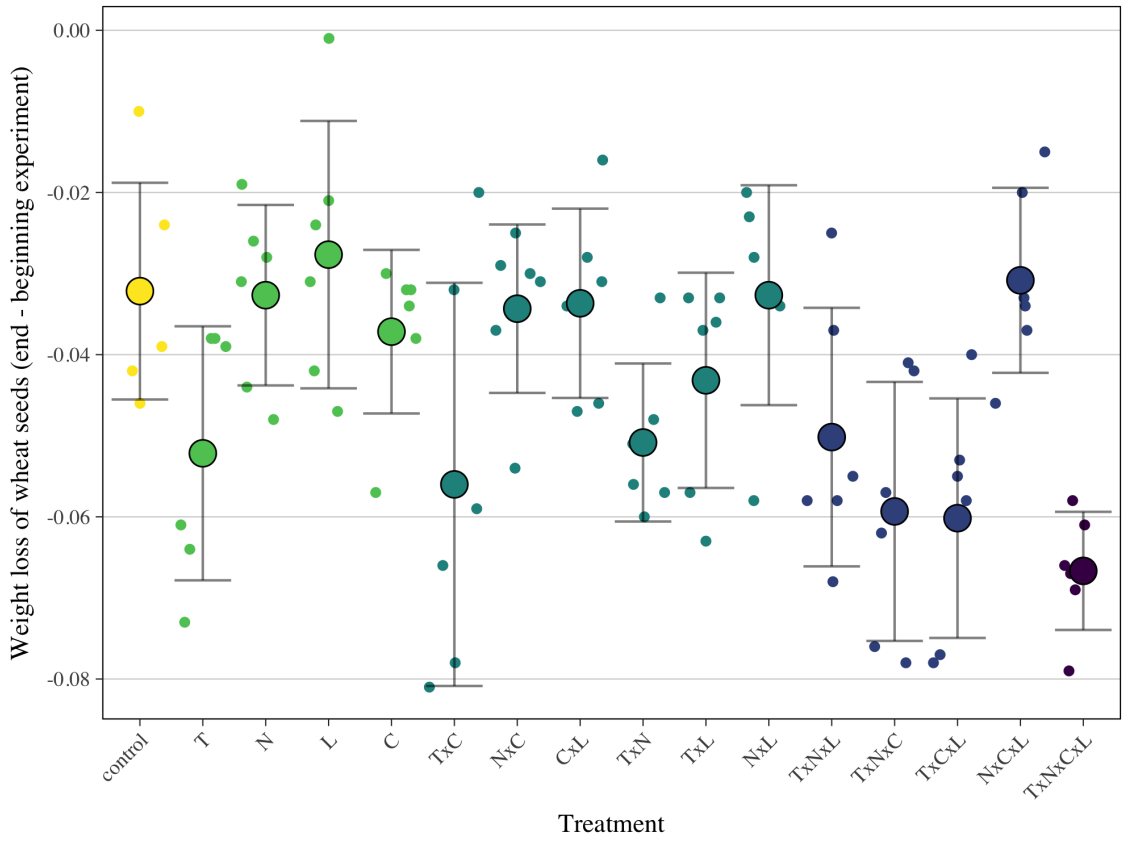
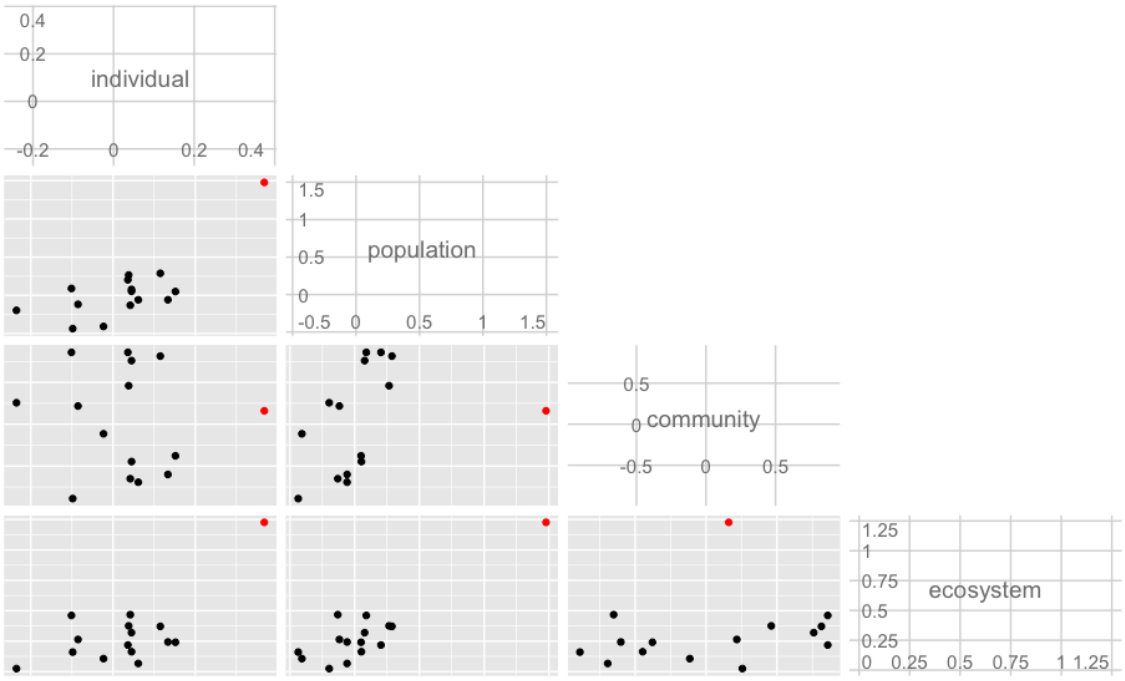



*Figure S28: Mean ± sd of the wheat seeds' weight loss between the end and the beginning of the experiment for each treatment. For the first eight days, all 96 microcosms were with control conditions (yellow; temperature at 20°C, N:P=40, organic matter 0.28g.L$^{-1}$ PPM, and 100% light availability. Then, disturbances were applied (vertical red line;* ***T****: 25°C,* ***N****: N:P=15,* ***C****: 0.56g.L$^{-1}$ PPM, and* ***L****: 70% light availability. The colors highlight the number of combined disturbances (yellow: control condition, light green: one, dark green: two, blue: three, and purple: four disturbances).*

*Table S26: Statisitcal analysis of the decomposition (ANOVA type III, Ajusted $R^2$ = 37.8%).*

| | Sum Sq | Df | F | Pr(>F) |
|---|---|---|---|---|
| (Intercept) | 0.006 | 1 | 31.5416 | <0.001 *** |
| Temperature (T) | 0.001 | 1 | 6.0968 | 0.016 * |
| Nutrient (N) | 0.000 | 1 | 0.0038 | 0.951 |
| Carbon (C) | 0.000 | 1 | 0.3810 | 0.539 |
| Light (L) | 0.000 | 1 | 0.3086 | 0.580 |
| T:N | 0.000 | 1 | 0.0256 | 0.873 |
| T:C | 0.000 | 1 | 0.0104 | 0.919 |
| N:C | 0.000 | 1 | 0.0847 | 0.772 |
| T:L | 0.000 | 1 | 0.1543 | 0.695 |
| N:L | 0.000 | 1 | 0.1543 | 0.695 |
| C:L | 0.000 | 1 | 0.0076 | 0.931 |
| T:N:C | 0.000 | 1 | 0.2439 | 0.623 |
| T:N:L | 0.000 | 1 | 0.0560 | 0.814 |
| T:C:L | 0.000 | 1 | 0.5641 | 0.455 |
| N:C:L | 0.000 | 1 | 0.0772 | 0.782 |
| T:N:C:L | 0.000 | 1 | 0.0008 | 0.977 |
| Residuals | 0.016 | 80 | | |

## Correlation with/without organic matter enrichment




Pearson's correlation coefficients between the levels of organisation with (upper, in red) and without the organic matter enrichment treatment "C" (lower, in black).

| | individual | population | community | ecosystem |
|---|---|---|---|---|
| individual | **1.00** | 0.76 | -0.07 | 0.69 |
| population | 0.49 | **1.00** | 0.34 | 0.89 |
| community | -0.15 | 0.62 | **1.00** | 0.22 |
| ecosystem | 0.27 | 0.51 | 0.38 | **1.00** |

# Chapter 4

# Manipulating organism-environment feedback strengths affects nonlinearity and hysteresis in a microbial predator-prey system.

**Abstract**

Organism-environment feedbacks have shown their importance in the context of global change mostly in theoretical ecology. These theoretical studies suggest that increasing the strength of feedback will 1) increase the likelihood of observing alternate stable states; 2) cause greater nonlinearity between an environmental change and ecosystem state; and 3) will increase the likelihood of hysteresis in response to an environmental change.

In an empirical test of the importance of organisms-environment feedback, we manipulated the metabolism - oxygen feedback strength in an aquatic heterotrophic tri-trophic community in microcosms. The manipulation consisted of five levels, from low to high feedback strength: free gas exchange (metabolism not strongly affecting environmental oxygen), regular addition of 200, 100, or 50mL of air, and no gas exchange. Additionally, to test for nonlinearity and hysteresis in response to environmental change, the microcosms experienced gradual temperature change from 15°C to 25°C, and then back to 15°C. We measured regularly the oxygen concentration in both head and liquid phases, the densities of the predator *Spathidium* sp., the prey (*Colpidium striatum* and *Dexiostoma campylum*) and the bacteria (initially *Serratia fonticola* and *Bacillus subtilis*).

Composition and dynamics of the communities showed evidence of multiple clusters (potentially alternate states), though the likelihood of residing in one or many of these clusters did not depend on the feedback strength treatment. In contrast, there was evidence of greater nonlinearity and greater hysteresis of the response to temperature change in treatments with stronger environment-organism feedbacks.

These empirical results are in broad agreement with the theory that stronger feedbacks increase nonlinearity and hysteresis, and represent one of the first empirical tests of such theory. Probing the mechanisms responsible for these empirical results with a mathematical model of the system could confirm the appropriate interpretation of the experiment, and thus this is a priority. Furthermore, experimentation with different community compositions and richness levels would allow tests of how biological structure might interact with feedback strengths to determine dynamics of ecological responses to environmental change.

# Introduction

Organism-environment feedbacks occur when the activities of organisms affect their environment, and simultaneously the environment affects the organisms (Hutchinson 1954; Tilman 1988; Jones *et al.* 1994; Naiman *et al.* 1999). In aquatic ecosystems, for example, organismal respiration acts to reduce the amount of dissolved oxygen, while the amount of dissolved oxygen affects organism vital rates (Breitburg *et al.* 1997; Fenchel 2005; Fenchel & Finlay 2008; Forster *et al.* 2012). Negative feedback loops tend to stabilize dynamics, for example by dampening fluctuations resulting from interactions, whereas positive ones can destabilize dynamics such that fluctuations increase in magnitude (Jones *et al.* 1994; Seto & Iwasa 2011). In networks, such as food webs, there are many biotic-biotic feedback loops (i.e., species interactions; when the activities of an organism affect the activities of another), and their strength has strong effects on system stability (Neutel *et al.* 2002).

Worldwide, all ecosystems types undergo changing environmental conditions which contribute to unprecedented faster rates of extinctions (Pimm *et al.* 1995). In these highly disturbed ecosystems, the feedback between the biota and its environment are disrupted (Naiman *et al.* 1999). Indeed, either the links within the food web can disappear (e.g., loss of predator with over-exploitation (Estes *et al.* 2011)) or fluxes can be drastically altered, which would affect the entire ecosystem (e.g., eutrophication with nutrients in excess (Scheffer *et al.* 2003)). Effects of climate change on ecosystems are complex as they are both top-down and bottom-up influences, and their feedbacks can be both positive and negative (Moorcroft 2003; Bony *et al.* 2006; Heimann & Reichstein 2008). Hence, understanding feedbacks in ecosystems is key for understanding ecosystem dynamics and how they respond to environmental changes.

Organism-environment feedbacks have interested ecologist as potential mechanisms behind the stability of ecosystems (Lotka 1925; Watson & Lovelock 1983). A well-known example is shallow lakes when the state of the ecosystem depends on organism-

environment feedback (e.g., Scheffer 1998; Scheffer & Nes 2007). In shallow lakes, the zooplankton controls the phytoplankton (top-down) and the fish abundances (bottom-up). Such regulation affects the environment by reducing the suspended particles, allowing light penetration, promoting the growth of submerged plants that provide refuges for zooplankton. This ecosystem state is mainly governed by a negative organism-environment feedback loop (zooplankton -(-)-> turbidity -(-)-> submerged plants -(+)-> zooplankton) that stabilise the system over time. When an excess of nutrients loads into the shallow lake, the phytoplankton growth is promoted and surpasses their consumption by zooplankton. This causes a bottom-up consequence on increasing the fish abundance, which affects the environment by increasing the suspended particles, decreasing the light availability, reducing the submerged plants, and zooplankton refuges. Additionally, the increasing abundance of fish promote phytoplankton growth by recycling nutrients, creating, therefore, a positive feedback loop (nutrients -(+)-> phytoplankton -(+)-> fish -(+)-> nutrients) that maintained the turbid environment.

Such shallow lakes can exhibit alternative stable states, nonlinearity and hysteresis. Alternate stable states can be observed when different system configurations are stable for the same environmental condition. For example, with the same nutrients concentration, both clear and turbid states can be observed. Nonlinearity reflects how the system configuration changes with variation in environmental input level; when extreme, nonlinearities are sometimes termed "tipping" points. In shallow lakes, the transition between two stable states is abrupt due to a shift in dominance among organisms (Scheffer *et al.* 2001), possibly when zooplankton top-down control is overwhelmed by phytoplankton growth. Hysteresis refers to a historical dependency of the system state (Holling 1973; May 1977). This phenomenon was observed after inconclusive conservation measures (i.e., stop of nutrients loading; e.g., Meijer *et al.* 1989), when the shallow lake remained turbid. It was only after a large decrease in nutrient concentrations that shallow lakes recover to a clear state. These types of system behaviour,

and the role of feedbacks in creating them, are exemplified in other systems such as deserts, coral reefs, woodlands, and the oceans (Scheffer *et al.* 2001).

Theoretical studies highlighted the importance of organism-environment feedback on diverse ecological research areas, such as niche construction (Jiang & DeAngelis 2013), competition (Golubski 2007), population extinction (Qin *et al.* 2017), metapopulations (Han *et al.* 2009), community structure (Seto & Iwasa 2011; Riegl & Piller 2000; Muthukrishnan *et al.* 2016), food web dynamics (Brown *et al.* 2004), and conservation/restoration (Suding *et al.* 2004). Given their importance in governing system dynamics and for how systems respond to environmental change, it is somewhat surprising that - to our knowledge - there are no experimental studies manipulating the strength of organisms-environment feedback and testing effects on multistability, nonlinearity, and hysteresis. Experimental manipulations of chemostat dilution rate come close, as dilution rate influences the effect of resource consumption on resource concentration (e.g., Fussmann *et al.* 2000). Dilution rate also influences mortality rates, however, and we are not aware of studies of effects of dilution rate on multistability, hysteresis, or nonlinearity.

Our study aimed to examine effects of the strength of organism-environment feedback on ecosystem dynamics and ecosystem response to environmental change. Small laboratory-based communities of aquatic microorganisms were a relevant and convenient study system, given the diversity of common ecological processes taking place (e.g., growth, death, consumption, competition, predation), the fast generation time of the organisms, and ease of monitoring and manipulation. The concentration of dissolved oxygen was the focal environmental variable, since this is a key environmental variable in aquatic ecosystems, and also as we conceived a method for manipulating the strength of the organisms-environment feedback involving oxygen, by altering how open the microcosms were to the surrounding atmospheric gases. When open to the surrounding atmosphere, consumption of oxygen by organisms has a weaker effect on

dissolved oxygen, since used oxygen can be quickly replaced from the surrounding atmosphere (which has constant ∼21% oxygen). When closed, consumption has a stronger effect, reducing dissolved oxygen concentration. Thus, by manipulating openness of the microcosm to the surrounding atmosphere, we manipulated the strength of the effect of organisms on their environment, and hence the strength of the feedback loop between organisms and their environment. We did not manipulate the other half of this feedback loop (the effect of dissolved oxygen concentration on the organisms) and furthermore did not have information on the dependence of organismal rates such as respiration, reproduction, consumption, and death on dissolved oxygen concentration (Figure 1).

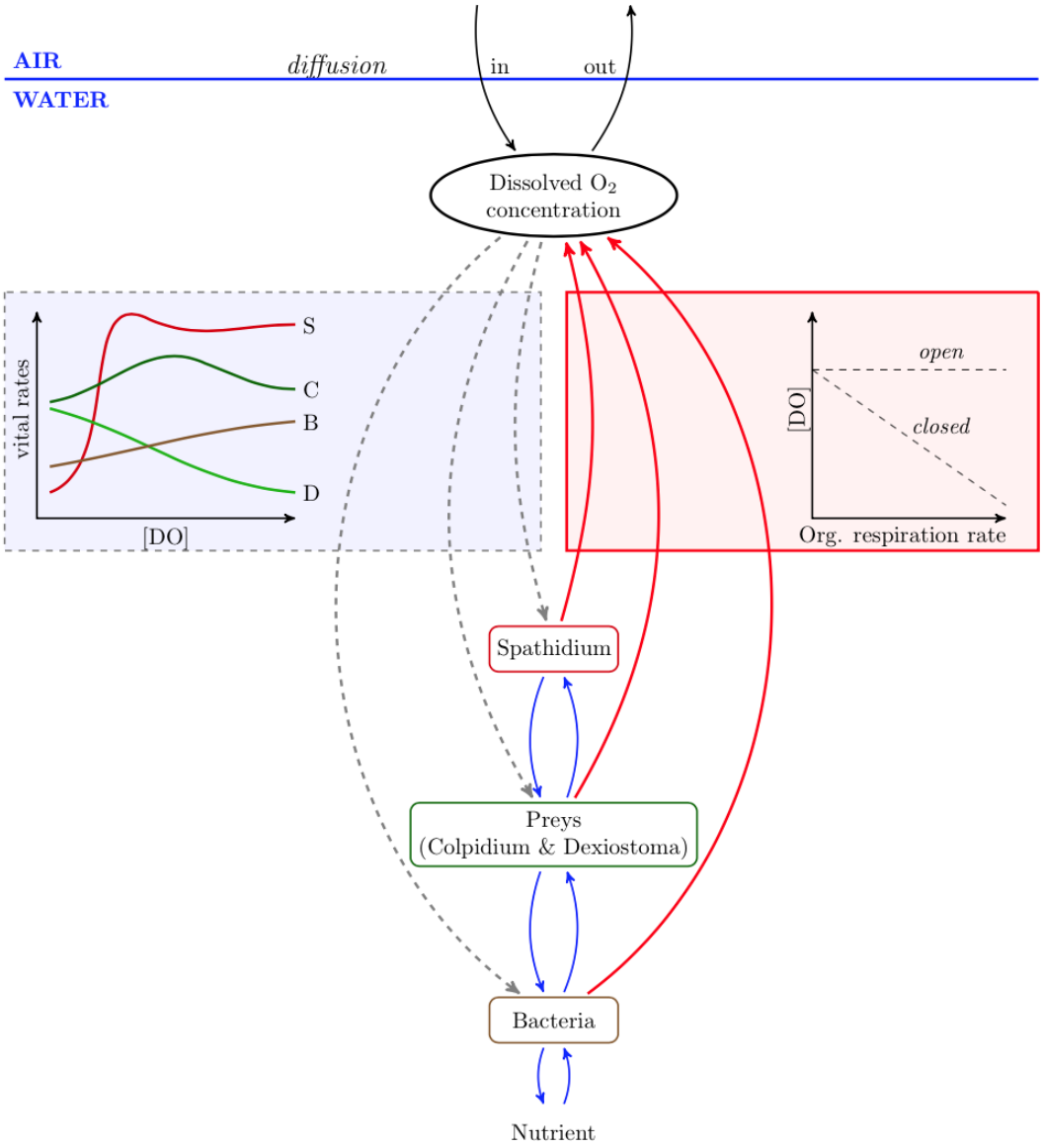



*Figure 1: Conceptual framework of the manipulation of oxygen in a predator-prey system. The negative organism-organism feedback loops (i.e., interspecific interactions) are represented by the blue arrows. The effect of oxygen on vital rates are represented in dashed grey arrows as the relationship were unknown. The effect of organismal respiration on dissolved oxygen in represented in red (all negative). With lower rate of oxygen flux into the water, the effect of respiration on dissolved oxygen (DO) increases; with high flux into the water the effect is decreased.*

We tested the following hypotheses based on general theoretical models of effects of feedback strength on ecosystem stability (Fussmann *et al.* 2000; Scheffer & Carpenter 2003; Rietkerk *et al.* 2004; Ibelings *et al.* 2007; Kéfi *et al.* 2016). Increasing the strength of feedback will: 1) increase the likelihood of observing alternate stable states; 2) cause greater nonlinearity between an environmental change and ecosystem state; and 3) will increase the likelihood of hysteresis in response to an environmental change. To examine hypotheses involving the response to an environmental change, and to examine hysteresis, we gradually increased and then gradually decreased the temperature of the microcosms.

# Material and Methods

## Experimental system

Microcosms were sterile 250 mL glass jars containing 100 mL Protozoan Pellet Medium (PPM) (Altermatt *et al.* 2015). Media consisted of 0.55 g of crushed Protozoan Pellets (Carolina Biological Supply Co., Burlington, N.C. USA) in one liter of Chalkley's medium, and then filtered using 0.45 µm before sterilization by autoclave. Two additional wheat seeds per microcosm provided a slow-release nutrient-source. Microcosms were placed in a dark temperature-controlled incubator.

The microbial aquatic community consisted of two bacteria species (*Serratia fonticola* and *Bacillus subtilis*), two bacterivorous prey species (*Colpidium striatum* and *Dexiostoma campylum*) and one predator species (*Spathidium* sp.). To avoid extinction caused by starvation, *Spathidium* sp. forms cysts and emerges when prey (preferably the smaller species; i.e., *Dexiostoma campylum*) increase in abundance.

We initiated the community with the bacteria grown at 37°C for 24 hours, and then added *Colpidium striatum* and *Dexiostoma campylum*. Before the addition, these prey species were grown in monoculture for seven days at 15°C to reach carrying capacity.

On day 0, we prepared the microcosms by combining 45 mL of *Colpidium striatum*'s culture (with ∼100 individuals per mL), 45 mL of *Dexiostoma campylum*'s culture (with ∼300 individuals per mL), and added 10 mL of *Spathidium* sp. at a density of 12 individuals per mL.

## Experimental design

To manipulate the strength of the organism-environment feedback loop, we altered the strength of the effect of organismal respiration on dissolved oxygen concentration. This was achieved by varying the rate of gas exchange between the atmosphere surrounding the microcosms (which was constant 21% oxygen) and the head space of the microcosm. Higher rates of gas exchange lead to weaker effects of organismal respiration on dissolved oxygen concentration, and a weaker feedback loop. Lower rates lead to stronger effects of respiration on dissolved oxygen, and a stronger feedback loop.

The rate of gas exchange was controlled by sealing each microcosm jar with a 3cm silicon stopper with two holes. The first hole contained a glass tube of 0.7 cm diameter to allow microcosm sampling with a Pasteur pipette and was sealed with a 0.7 cm silicon stopper (to prevent gas flux) or a sponge (to allow gas flux). The second hole contained a hypodermic needle fitted with a 0.4 cm silicon stopper. This apparatus allowed us to implement five oxygen exchange treatment levels: continuous exchange (by putting a sponge in the glass tube), exchange of 50 mL, 100 mL or 200 mL air every second day (via the needle, and with a stopper in the glass tube), or no exchange (a stopper in the glass tube). Preliminary experimentation validated that these treatment levels had expected effects on dissolved oxygen concentrations (Figure S1).

To study the response to an environmental change, all microcosms were exposed to the same temperature regime: an increase of 0.7°C every two days for 30 days, constant 25°C for a week, and then a decrease of 0.7°C every two days for 30 days.

## Measured variables

All measurements were made every two days.

**Oxygen measurement.** The percentage of oxygen (% $O_2$) was measured using non-invasive chemical-optical sensing (Fibox 4trace, PreSens, Germany; Altermatt *et al.* 2015). This method involved to fix the sensors inside the microcosms to the vessel walls in the head and liquid spaces (at the same depth in all microcosms) which allowed the measurements with an optical probe from the outside. Oxygen measurement was made before any others, and without moving the microcosms to avoid any effects of movement on dissolved oxygen concentration.

**Predator density.** We estimated the predator density by counting the number of *Spathidium* sp. individuals in 1 mL by eye under a dissecting microscope.

**Preys density with video analysis.** To estimate the prey density, we used video analysis (Pennekamp *et al.* 2015). We placed in a custom counting chamber 700 µL of the 1 ml previously used to count the predators and made a 5 second video at 25 frames per second of ∼50 µL of the 700 µL using a camera (Hamamatsu Digital camera C11440) attached to a microscope (Leica M205C, 0.63X) and the software (HCImage Live version 4.0.6.3). The videos were analysed using `BEMOVI` package; this software isolates moving particles (here the ciliates), reconstructs their trajectories and assigns trajectories to species based on morphological traits using RandomForest classification (Pennekamp *et al.* 2017).

**Bacteria density with a flow cytometer.** We diluted 20 µL from the samples in 160 µL of filtered Ultra-Pure water and 20 µL of a ten-fold dilution of SYBER Green. This mixture was incubated at 37°C for 15 minutes in the dark. The flow cytometer was run with following parameters: volume sample 30 µL; medium fluid speed; FSC-H threshold of 20'000 and SSC-H threshold of 400.

*Table 1: AIC of polynomial regressions on the time series of each measured variable. Forward selection based on Likelihood Ratio Test (significant difference is shown by the letters).*

| | Oxygen $_{liquid}$ | Bacteria | Preys | Predator |
|---|---|---|---|---|
| Linear | $4843.23^a$ | $486.67^a$ | $2387.43^a$ | $567.23^a$ |
| Quadratic | $4727.55^b$ | $361.37^b$ | $2306.54^b$ | $\mathbf{452.63}^b$ |
| Cubic | $\mathbf{4633.15}^c$ | $225.06^c$ | $2090.72^c$ | $453.90^b$ |
| Quartic | $4632.55^c$ | $154.26^d$ | $\mathbf{2079.66}^d$ | - |
| Quintic | - | $\mathbf{93.41}^e$ | $2081.62^d$ | - |
| Sextic | - | $92.32^e$ | - | - |

## Statistical analyses

**Alternate states.** Evidence for alternate states was sought via cluster analysis, and evidence for effects of the feedback treatment on alternate states by analysis of the distribution of replicates among any clusters found. Clustering of final community composition (average over last five days, i.e., three samples) and of dynamical pattern was assessed. The dynamical pattern of each replicate and each variable (i.e., the abundance of a species and the oxygen concentration) was quantified by the coefficients of polynomial regression of a variable against time. Forward selection from a linear regression to a sextic regression (polynomial of degree 6) occurred until the absence of difference between two models (Table 1; Zar (1996)). As the complexity of the polynomial increased, the modelled dynamics showed more fluctuations (e.g., Angert *et al.* 2007). To minimise the number of parameters (coefficients of the regressions) for the cluster analysis, we performed a PCA and removed similar coefficients, resulting in eight coefficients describing the dynamical patterns, two for each variable (Figure 2).

The presence of multiple clusters was assessed by comparing the likelihood of a multivariate mixture model with more than one cluster to such a model with only one cluster. Multivariate mixture models were implemented with functions in the `mclust` R package (Fraley & Raftery 2002; Fraley *et al.* 2012). Variables were BoxCox transformed prior to fitting the mixture models. Dependence of community occupancy in single or multiple states on the feedback strength treatment was tested by logistic

regression, with one or more than one state as the binary response variable and the gas exchange treatment as the explanatory variable.

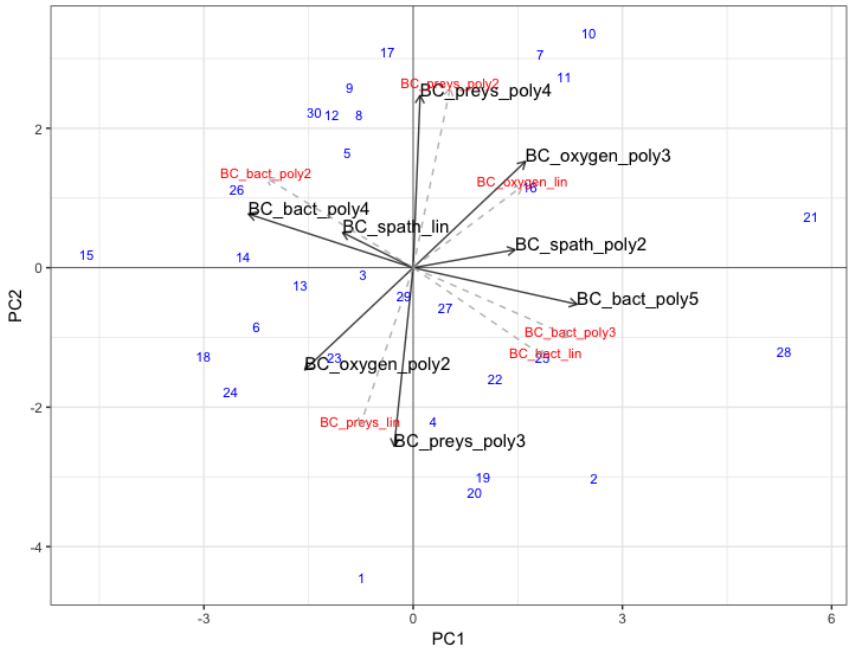



*Figure 2: Reduction of the number of coefficients describing the community dynamics. When two coefficients were similar (i.e., same direction) we kept coefficients of higher polynomial, and then we removed the coefficients in red.*

**Nonlinearity.** For each microcosm, separately for the temperature increase phase and temperature decrease phase, we calculated the nonlinearity of the relationship between dissolved oxygen concentration and temperature. Nonlinearity was calculated as the root mean square difference between fitted values of a linear model and a non-linear model (Emancipator & Kroll 1993). A generalised additive model (GAM) fitted using the default options of the gam function in the `mgcv` R package was the nonlinear function. Response (oxygen concentration) data were not standardised in order to obtain a measure of absolute rather than relative nonlinearity (Emancipator & Kroll 1993). Linear models were used to test for an effect of the gas exchange treatment on this measure of nonlinearity. We apply this method only to the dissolved oxygen

concentration, as it summarises the state and effect of the ecological community.

**Hysteresis.** To estimate hysteresis in response to the increasing then decreasing temperature experienced by each of the microcosms, we compared the DO concentration measured at a particular temperature during the temperature rise to the oxygen concentration measured at the same temperature during the temperature decrease. A close match between oxygen concentrations in the increasing and declining temperatures would indicate lack of hysteresis; a difference would indicate hysteresis. For each microcosm, we calculated the $R^2$ of the relationship between these paired oxygen concentrations, and also the mean absolute difference between the paired oxygen concentrations. We used linear models to test for an effect of the gas exchange treatment on each of these response variables. We apply this method only to the dissolved oxygen concentration, as it summarises the state and effect of the ecological community. Analyses were performed using the statistical software R (R Core Team 2016).

# Results

## General ecosystem dynamics

All communities started with around 10% dissolved oxygen (DO), $1x10^6$ bacteria per ml, 1000 prey per ml, and 10 predators per ml (Figure 3). Prey density then decreased rapidly, coincident and presumably due to increasing abundance of the predator, and coincident with increasing DO concentration (Figure 3). After day 10 - 20, the dynamics started to diverge among the communities: some with predator and prey becoming very rare for the remainder of the experiment (e.g., community 129), some with prey (mainly *Colpidium striatum*) increased to high abundance and predators remaining at relatively low abundance (e.g., community 126), and some with both prey and predators increasing in abundance towards the end of the experiment (e.g., community 112).

All of the open microcosms showed a gradual increase in DO up to just below

20% and equilibrated with the oxygen level in the head space. Oxygen dynamics in the other treatments, in which oxygen flux was restricted, varied among replicates: some showing dynamics very similar to those of the open treatment, and some showing declines in DO later in the experiment.

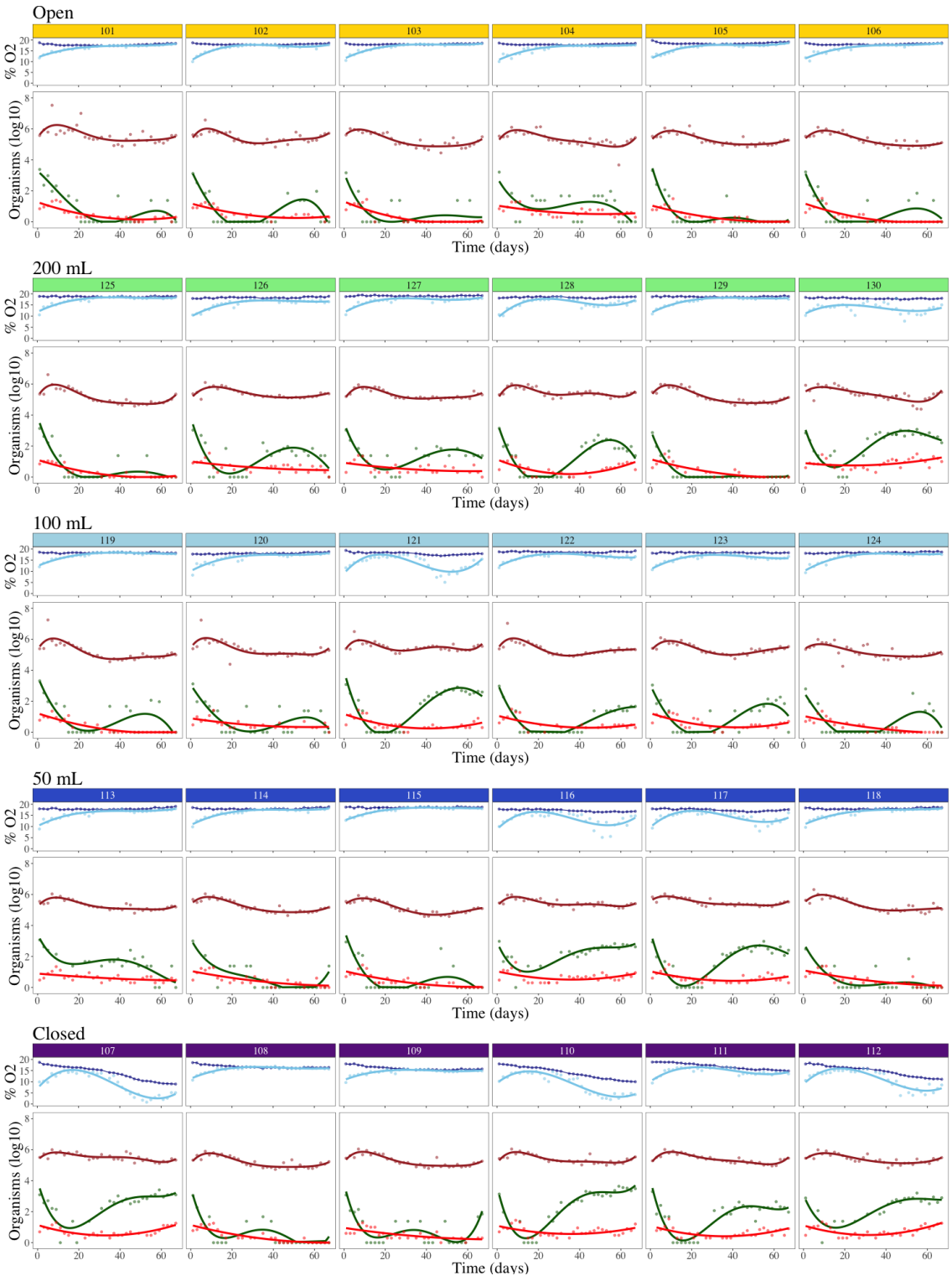



*Figure 3: Dynamics of % $O_2$ in the liquid (light blue, with a cubic polynomial regression), in the head space (dark blue), bacteria density (brown, with a quintic polynomial regression), preys (green, with a quartic polynomial regression) and Spathidium sp. (red, with a quadratic polynomial regression) for each microcosm (from 101 to 130) according to the gas exchange treatment: 101-106: OPEN, 125-130: +200 mL, 119-124: +100 mL, 113-118: +50 mL, and 107-112: CLOSED.*

## Alternate states

Based on BIC criterion, the multivariate cluster analysis of all communities showed that model with four (for final abundances, Figure 4b) and two clusters (for dynamics, Figure 4d) was more plausible than one with only one cluster. The comparison between the null hypothesis (i.e., one cluster) showed a weak statistical difference (final measurements: $\text{logLik}_{MOD4} - \text{logLik}_{MOD1} = 37.85887$, df $= 26$, $P = 0.062$; coefficients: $\text{logLik}_{MOD2} - \text{logLik}_{MOD1} = 99.52611$, df $= 81$, $P = 0.079$).

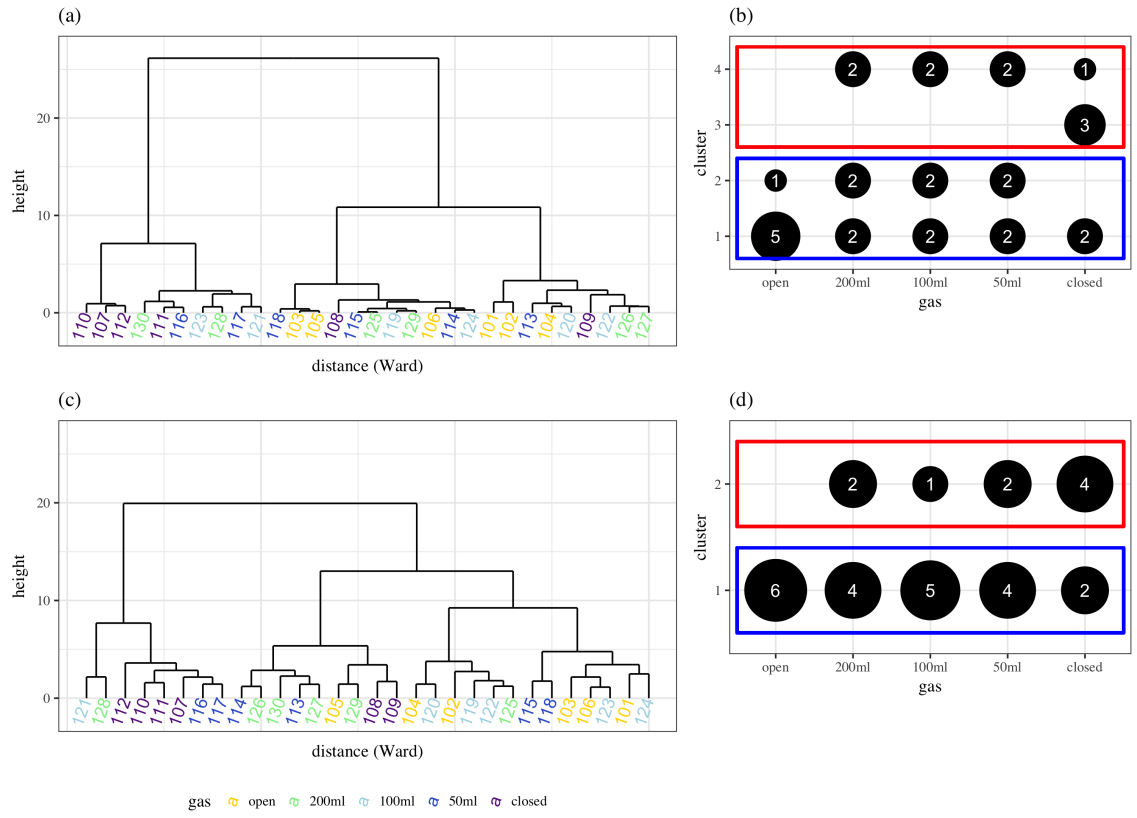



*Figure 4: Multivariate cluster analysis on the two response variables. Final measurements (a and b) clustered using a hierarchical cluster analysis (a) and model based cluster analysis (b). Coefficients of the dynamics (c and d) clustered using a hierarchical cluster analysis (c) and model based cluster analysis (d).*

Four clusters described variation in final abundances among microcosms. The first

cluster, grouping 13 microcosms, was characterised by low final densities of *Spathidium*, preys and bacteria, and high dissolved oxygen concentration. The second cluster (7 microcosms) was similar to the first cluster except with higher final densities of Spathidium and bacteria. The third cluster (3 microcosms) was the most different from other clusters, with very lower dissolved oxygen concentration, higher final densities of *Spathidium*, preys and bacteria. The fourth cluster (7 microcosms) had intermediate values between the clusters 1-2 and 3: higher final densities and lower dissolved oxygen concentration than the clusters 1 and 2 but lower final densities and higher dissolved oxygen concentration that the cluster 3.

Two clusters described the dynamics. The first cluster (21 microcosms) characterised microcosms with few fluctuations whereas the second cluster (9 microcosms) characterised microcosms that fluctuated over time. In other words, the second cluster highlighted microcosms with a recovery of the predators and preys, and a decrease in dissolved oxygen concentrations.

From these two response variables, we characterised mainly two states: 1) extinctions or close to extinction of the predator-prey system with less fluctuation of the dissolved oxygen due to its stabilisation; and 2) recovery of the predator-prey system with more fluctuation of the dissolved oxygen concentration and bacteria densities.

Note that these two main states resulted in grouping the clusters 1 and 2, and the clusters 3 and 4 for the analysis of the final densities, although the cluster analysis never suggested these two possible clusters. Indeed, when the model was "forced" to create two clusters, it grouped the clusters 1-3-4. Nevertheless, from the comparison of the two response variables (final measurements and coefficients of the dynamics), this clustering made sense as the microcosms were clustered similarly except for one community (microcosm 123).

There was no evidence that probability of a community being in any particular state or set of states was influenced by the gas exchange treatment (logistic regression

with likelihood ratio test has $P = 0.11$ for the final densities; $P = 0.08$). However, all microcosms that experienced open gas exchange were clustered in a unique cluster with lower final densities of *Spathidium*, preys and bacteria and higher final dissolved oxygen concentrations.

## Nonlinearity of environmentl-system state relationship

During the gradual temperature increase of the first half of the experiment, nonlinearity was increased by increased in how closed was the gas exchange treatment, with greater nonlinearity in the closed treatment (Figure 5; ANOVA, $F_{4,25} = 3.04$, $P = 0.04$; regression $F_{1,28} = 8.83$, $P = 0.006$). During the second half of the experiment when temperature was decreasing, there was a similar trend towards greater nonlinearity in more closed treatments, but lower statistical significance (Figure 5; ANOVA, $F_{4,25} = 1.09$, $P = 0.38$; regression $F_{1,28} = 4.63$, $P = 0.040$).

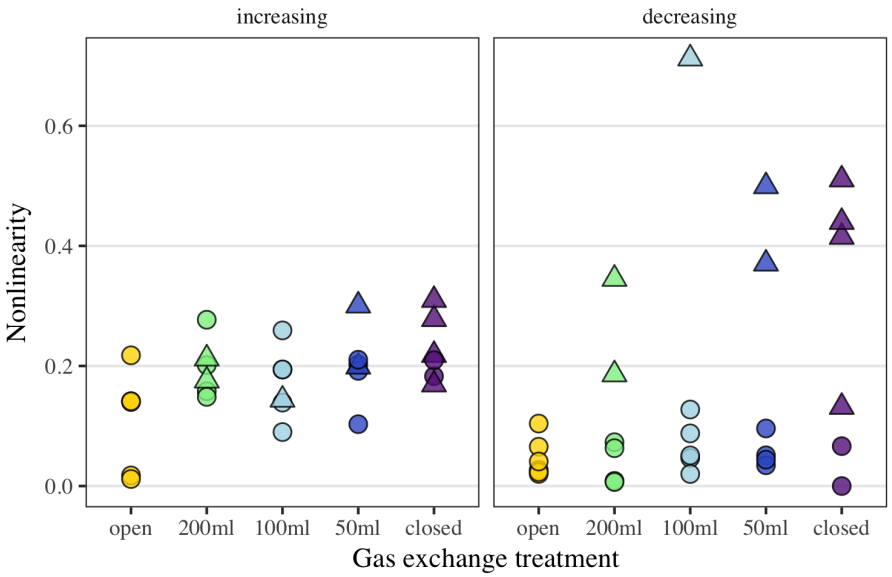



*Figure 5: Effects of gas exchange treatment on nonlinearity of relationship between dissolved oxygen concentration and temperature, during the first half of the experiment when temperatures were increasing, and the second half when they were decreasing. The symbols highlight the clustering (circle for the cluster 1 and triangle for the cluster 2 in Figure 4d).*

During the gradual temperature decrease, the moderate evidence of nonlinearity between treatments did not seem surprising as we already did not observe a strong difference of the treatments for the alternative states (cluster analysis). However, we observed that the nonlinearity differed the two clusters observed for the alternate states (Wilcoxon test; $P < 0.001$). Indeed, the nonlinearity was higher in the microcosms with higher final densities of *Spathidium*, preys and bacteria and lower dissolved oxygen concentrations (triangles in Figure 5 represented the red cluster in Figure 4d).

## Hysteresis

Gas flux treatments associated with stronger feedbacks caused greater hysteresis, according to both measures of hysteresis (Figure 6). When coded as a categorical explanatory variable, there was no apparent effect of the gas flux treatments on either measure of hysteresis ($R^2$: ANOVA, $F_{4,25} = 2.48$, $P = 0.07$; Mean difference: ANOVA, $F_{4,25} = 2.00$, $P = 0.13$). When the gas flux treatment was coded as a continuous explanatory variable with five equally spaced levels, the linear regression of $R^2$ versus gas exchange treatment level had a negative slope ($P = 0.03$) and the linear regression of mean difference versus gas exchange treatment had a positive slope ($P = 0.06$). There was a considerable difference among replicates, particularly in the closed gas exchange treatments (Figure 7).

Additionally, we analysed both measurements ($R^2$ and mean difference) according to their attribution to a particular cluster (Figure 4d) using a Wilcoxon test (unbalanced data). We did not observe difference of $R^2$ between the two clusters ($P = 0.59$). Whereas the mean difference differed between the two clusters observed for the alternative states ($P < 0.001$). Indeed, the mean difference was higher in the microcosms with higher final densities of *Spathidium*, preys and bacteria and lower dissolved oxygen concentrations (triangles in Figure 6 represented the red cluster in Figure 4d).

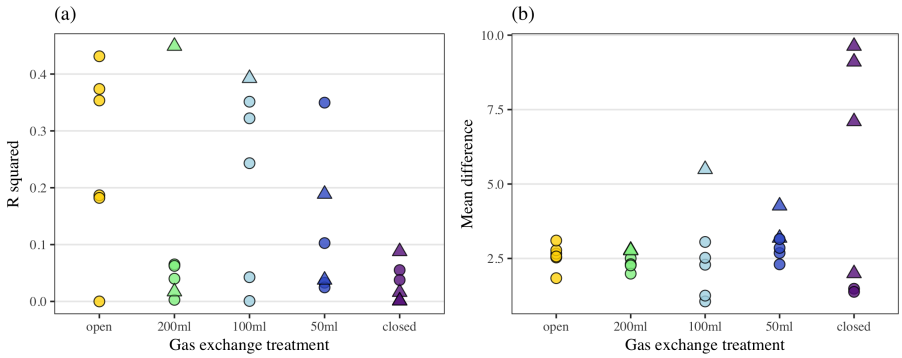



*Figure 6: Two measures of hysteresis for each microcosm according to their gas exchange treatment. (a) The $R^2$ depicts the linear relationship of dissolved oxygen between the increasing and decreasing temperature phase and (b) the mean difference of dissolved oxygen at an equivalent temperature. The symbols highlight the clustering (circle for the cluster 1 and triangle for the cluster 2 in Figure 4d).*

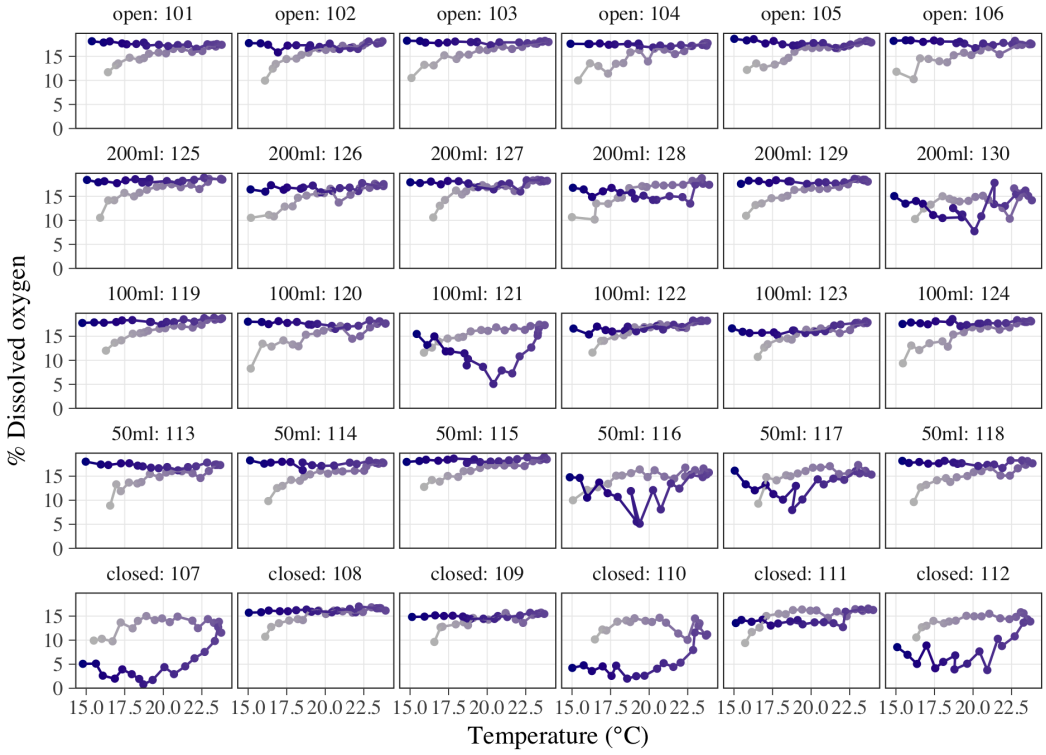



*Figure 7: Dissolved oxygen across the temperature and temporal gradients. Each row shows a gas exchange treatment with its six replicates. The temporal change is represented by the gradient from grey to blue.*

# Discussion

The experimental ecosystems in our study exhibited greater nonlinearity in response to environmental change and greater hysteresis when organism-environment feedbacks were stronger. These findings support broad theoretical predictions about the importance of system feedbacks in the dynamics of ecological responses to environmental change.

## Alternate states

From the middle of the experiment when the temperature started to decrease, we observed two states (i.e., bistability) in our communities: either extinction of the system along with high dissolved oxygen concentration or recovery of the predator-prey system along with lower dissolved oxygen concentration. The recovery of the predator-prey' system was observed mainly in the systems with the manipulated gas exchange. This result is somehow surprising, as we expected predator-prey dynamics in the open system (e.g., Fussmann *et al.* 2000; Petchey 2000; Shertzer *et al.* 2002).

Four elements in our experiment could explain these results (Figure 8). First, micro-organisms can survive in, even thrive in, a very large range of environmental conditions (Fenchel *et al.* 1997). Especially, in extreme conditions such as acidity (Packroff 2000), temperature (Laakso *et al.* 2003) and anoxia (Bernard & Fenchel 1996). *Colpidium striatum* and *Serratia fonticola* are one of the micro-organisms' species that can grow either in aerobic or anaerobic condition (Fenchel 2005). Thus, there may be weak effects of dissolved oxygen concentration on these organisms vital rates, and thus generally weak organism-environment feedback (though still some change caused by the experimental manipulation). Second, the oxygen limitation might have impacted *Spathidium* sp. and therefore relax the predation on the preys, allowing the preys to recover. Even though it is not known that *Spathidium* is affected by low oxygen levels (An-

drushchyshyn *et al.* 2003). Third, species have a different optimal temperature, and combined this feature with the oscillations observed in a predator-prey system. This hypothesis would allow some *Colpidium striatum* to survive at a higher temperature, and to benefit from an optimal temperature (unpublished data) and therefore to grow faster than their consumption by *Spathidium* sp. More generally, interspecific variation in these responses would affect the ecosystem level effect of oxygen concentration on organismal respiration, and thus the overall organism-environment feedback. And fourth, the presence of cysts could also buffer the dependence to preys' availability (Brown *et al.* 2004), even if cysts were also abundant in the open systems.

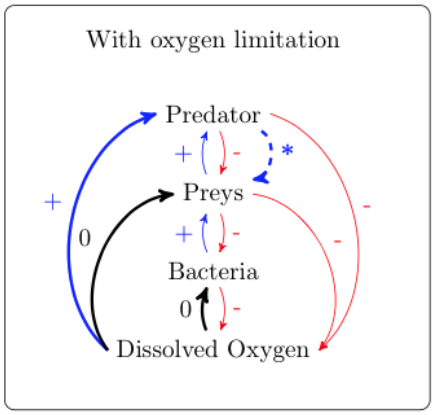



*Figure 8: Mechanisms to explain the recovery of predator-preys' system: 1) no effect of oxygen limitation on prey (Colpidium striatum) and bacteria (S. fonticola) and 2) the negative feedback loop between dissolved oxygen and predator which lead to a predation relax on their preys (*).*

We can also question the experimental design, whether the limitation of oxygen was constraining enough. Indeed, a last possible explanation is that the limitation in oxygen (especially the addition treatment) was not stressful enough to have a strong influence on the dynamics. Indeed, we could not see differences in the dissolved oxygen between the open system and the three treatments with air addition. Nevertheless, we could see some recovery of predator and/or prey (for example, the microcosms 130 (+200mL), 121 (+100mL) and 116 (+50mL)). Therefore, for the same environmental

conditions (i.e., oxygen in the liquid phase), we could observe bistability with the two states (extinction or recovery). Bistability has been previously shown, theoretically, in the arid ecosystem with self-organised patchiness (Scheffer *et al.* 2003; Rietkerk *et al.* 2004) and in the cell signalling research (Angeli *et al.* 2004). In both cases, authors concluded that when the strength of the feedback increases, the bistability arose (Angeli *et al.* 2004; Kéfi *et al.* 2010).

## Nonlinearity

An essential aspect of complex adaptive systems is nonlinearity, leading to historical dependency, multiple possible outcomes of dynamics (e.g., alternative stables states), and qualitative shifts in system dynamics (i.e., catastrophic shift) (Levin 1998). However, in our experiment, we highlighted that nonlinearity might be driven internal processes caused by alternative states. Indeed, nonlinearity would involve a biological and a physical process: the consumption of oxygen by organisms and the oxygen diffusion between the liquid and the head spaces.

During increasing temperature, the oxygen level in the liquid phase increased until it equilibrated with the oxygen level in the head space, therefore the diffusion was greater than the consumption. We found a difference between the gas exchange treatment due to the differences in the maximum oxygen levels reached in the liquid phase (see also Figure S1). During decreasing temperature, the nonlinearity depended on the relative importance of consumption to the diffusion. When the predator-prey system recovered, the consumption was greater than the diffusion. This pattern was especially pronounced in the closed systems. Whereas, in microcosms with extinctions or in open systems, oxygen levels were stable (i.e., linear) over temperature decrease due to either the absence of consumption (extinction of the system) or the consumption was counterbalanced by diffusion. Therefore, the nonlinearity depended greatly on the system state.

## Hysteresis

Hysteresis, or memory effect, is a characteristic of regime shift that describes the dependence of a system state on its history. This phenomenon can be due to the presence of feedback that stabilises the states with biological, physical and chemical mechanisms (Scheffer *et al.* 2001), or due to the time delay of organisms in response its environment (Eurich *et al.* 2005).

In our experiment, hysteresis highlighted the difference of system state for identical temperatures. Hysteresis was found in systems where the predator and prey persisted, mainly due to the greater difference at intermediate temperature due to two different system states: 1) when the predator and preys were decreasing either decreasing (with increasing temperature), and 2) when the preys recovered (with decreasing temperature). Therefore, in our experiment, hysteresis seemed mainly driven by biological processes. However, it is difficult to disentangle the effect of oxygen, temperature and time as all of these external factors occurred simultaneously.

## Implication of this study

This project could also question the role of oxygen as a limiting resource when the system is closed. In a review of plant-soil feedback systems, the feedback strength is expected to diminish as the resources become less limiting (Revillini *et al.* 2016). Therefore, with an open system, the dissolved oxygen was constantly renewed by diffusion; thus, the bacteria would be the limiting resource of the food web. Whereas, in a closed system, the dissolved oxygen could become the limiting resource in the community, and therefore changed the dynamics in the ecosystem. While oxygen is rarely considered as a limiting resource, a short or prolonged absence of gas exchange might have a profound effect on the food web dynamics. Ecosystems subjected to ice cover and/or stratification are experiencing similar conditions and it would be interesting to

observe these alternative states in natural ecosystems. Already observed, Bush and collaborators (2017) observed that feedback between biochemical processes and microbial communities might be responsible for the shift from oxic to anoxic conditions (Bush *et al.* 2017).

Overall, a great deal of research remains to be done. First, a theoretical model of our system, ideally parameterised with empirical data about organisms affect on their environment, and the effect of the environment on organisms, would allow assessment that we are observing the predicted patterns (i.e., greater nonlinearity and hysteresis) for the right reasons. Second, understanding the importance of organisms-environment feedback strengths in the context of other types of environmental change than temperature, and when multiple simultaneous changes occur, is a priority. Finally, the effects of biodiversity on the influence of organism-environmental feedbacks are important to understand and predict, species richness and composition may both drive and respond to environmental change.

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# Supplement

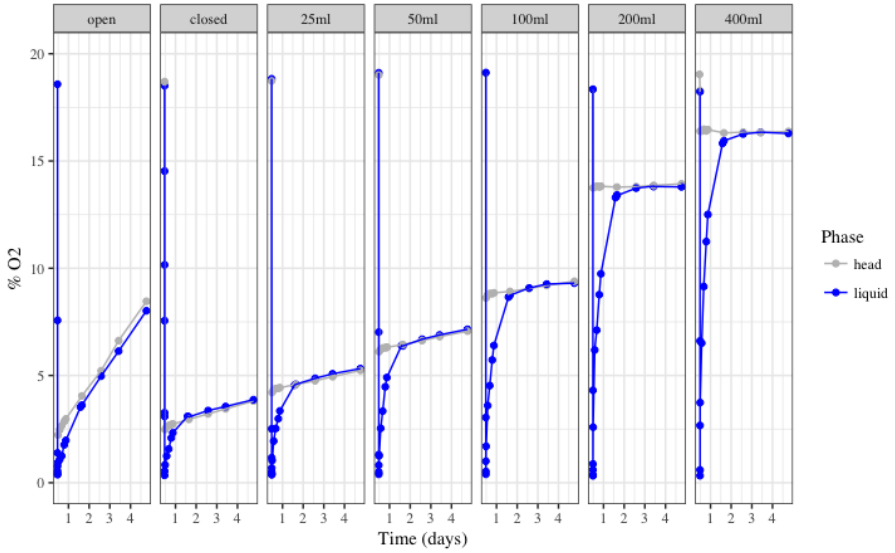


*Figure S1: Gas exchange manipulation method. First, oxygen was removed from the water using N2. Then, we followed the recovery of oxygen in the liquid (blue) and head (grey) spaces.*

# Chapter 5

# General discussion

Natural systems are often considered as complex adaptive systems (Levin 1998). Therefore, it was primordial to bridge different fields of research in ecology to picture the consequences of multiple environmental changes and of the feedback between organisms and their environment. The goal of this Ph.D. thesis was to provide empirical pieces of evidence regarding 1) the consequences of multiple global change drivers and 2) the consequences of organism/community – environment feedback strength on microbial aquatic communities. These two central research questions have significant implications regarding the predictability of natural systems while the pressure on these systems is increasing.

In this general discussion, I discuss my research in the framework of the five challenges presented in the general introduction (stability, multiple drivers of global change, up-scaling, predictability, and feedback) and highlight how these challenges are and have to be interconnected to provide insights of global change consequences on ecosystems.

## Stability

To characterize how an ecosystem variable responds to multiple environmental disturbances I used three stability measures (resistance, resilience and return time) in a temporal framework (short-, medium- and long-term response respectively). Indeed, the resistance described the maximum amount of change observed within three days after the perturbation. From this value, I estimated the speed of recovery (i.e., "engineering" resilience). And the return time was possibly observed when the ecosystem variable returned to the control range.

This method was possible due to one particular disturbance that had a rapid and large effect on the resistance of the ecosystem variable studied. Indeed, this disturbance – an increase of organic matter – likely enhanced the rapid bacterial growth

which then caused the observed depletion of the oxygen in the system. Following this increasing bacterial growth, their consumption by the consumers of the community allowed recovery of oxygen concentration towards undisturbed condition oxygen levels. Therefore, the effect of this disturbance on dissolved oxygen could be considered as a pulse disturbance.

I attempted to apply this method on other ecological variables (e.g., population dynamics in Chapter 3) but I did not observe strong results mainly because populations did not respond as quickly to the organic matter enrichment as the dissolved oxygen. Indeed, with this enrichment, the populations increased in abundances and the maximum amount of change (i.e., resistance) was not observed within three days. Additionally, this increase in abundance was maintained over time, with little or no recovery towards the abundances observed in the control microcosms. For the population dynamics, the organic matter increase could be considered as a press perturbation. Therefore, while stability measures can describe global responses to perturbations, it is also important to consider the reaction time of disturbance on the organisms, its implication (pulse *vs.* press disturbance), and adapt the analyses according to it. For this reason, in Chapter 3, while analysing results of the same experiment as in Chapter 2, I decided to analyze all the variables – from individual traits to ecosystem functions – with an identical manner across levels using a generalized additive model that was able to capture the main effects and interaction terms while taking into account the temporal change.

Ecologists (theoreticians and empiricists) study the stability of ecosystem differently when facing disturbances (Arnoldi *et al.* 2016). Theoreticians focused on the description of long-term recovery with the asymptotic rate of recovery (i.e., when the system is close to equilibrium), whereas empiricists focused the description of short-term responses such as resistance and immediate rate of recovery (Chapter 2). It appears more relevant, for practitioners, to focus on short-term indicators of stability

as natural ecosystems are most likely subjected to consecutive perturbations and it is better to know earlier if the system will recover to be able to engage restoration measures.

## Multiple drivers

Ecosystems are subject to simultaneous multiple environmental drivers. In this thesis, I highlighted three major results: 1) the dominant effect of one disturbance observed at three levels of organisation (individual traits, population dynamics and ecosystem functions), 2) the detrimental accumulation of disturbances on two stability measures (resistance and return time), and 3) the small proportion of detected interactions between environmental disturbances across levels of organisation. These results give insights for further research on multiple environmental disturbances.

The first insight is about the nature of the relationship between the response variables and the number of environmental disturbances. I observed opposite signs between the 2-, 3- and 4-way interactions terms, indicating reversal interactions. This pattern that has been particularly observed on the overall effect of multiple environmental drivers at the community level (Chapter 3). This observation might highlight the nature of the relationship between the response variable and the number of environmental drivers. Indeed, if the relationship is linear, the effects of cumulative environmental disturbances should be additive; whereas if the relationship is non-linear, the interactions between environmental disturbances should be detected. According to the type of relationship, interactions could be expected: an increasing exponential relationship to describe synergistic interactions, a decreasing exponential relationship for antagonistic interactions. These two specific relationships would consider that all interactions act in the same direction. If not, we could expect that the relationship would be a saturating curve, therefore describing reversal interactions that counteracted the interactive effect

of lower complexity of interaction.

The second insight is about the detection of interactions. I highlighted that the interactions were rare and the linear detrimental cumulative effect on stability measures. Both of these results were in favor of the additive hypothesis (i.e., linear relationship). However, this result contradicts the overall observation, from meta-analyses, that antagonistic interaction would be more likely to occur in natural systems (Jackson *et al.* 2016). Therefore, we can question our choice of level for the different environmental disturbances, or whether the publication bias against non-significant results (Lederman & Lederman 2016) would underestimate the absence of interactions between disturbances.

For future research, two options should be considered. The first option would be to increase the number of replicates to be able to detect interactions with the increase the statistical power. The second option, ideal but more complex logistically, would be to invest the research at a larger scale using experiments globally coordinated. Such experiments have already been set up to study the effects of nutrients ("NutNet") or dispersal ("dispNet") on different ecological systems. Therefore, an experiment investigating different ecological systems with an intensity gradient of multiple disturbances would a provide global effect of global change. Additionally, such experiments should prioritise three aspects: 1) a mechanistic understanding of multiple environmental on individual organisms 2) to up-scale these impacts across biological levels of organisation and 3) examine the context-dependent changes in disturbances responses regarding the ecological system studied (Griffen *et al.* 2016).

# Up-scaling

To observe responses of multiple environmental disturbances across four scales/levels of ecological organisation (individual, population, community and ecosystem), I used a similarity/dissimilarity approach. I highlighted those individual traits, population

dynamics and ecosystem functions had similar responses to disturbances. However, the similarity was mainly due to the average positive effect of organic matter enrichment.

With such experimental study, a priority for future research would be to explore the up-scaling of multiple environmental disturbances and investigate how much information on a lower level of organisation (individual traits) would explain a higher level of organisation (population dynamics or ecosystem functions), and therefore provide a more mechanistic approach instead of the correlational proposed in Chapter 3.

Some concern was raised about up-scaling responses across levels of organisation (Longo *et al.* 2012). Indeed, the authors based on distinctions between a scale that refers to a quantity varying in magnitude (e.g., space, time, energy) and a level that refers to a qualitative change between objects. In my research, I considered different variables to characterise levels of biological organisation, the community structure and composition was mainly based on the identity of the species; whereas individual traits, populations abundances and ecosystem functions might be seen as a productivity or energy transferred. Therefore, this difference might explain why I did not see a correlation in response to multiple disturbances between the community level and the others.

## Predictability

With increasing pressure on natural ecosystems, the predictability of how multiple environmental disturbances impact those ecosystems is an essential feature for conservation measures, management of natural resources and policy decisions (Houlahan *et al.* 2017). In this thesis, I investigated whether including interactions (even though they were not common – Chapters 2 & 3) affected the hindcast predictability (i.e., how well an observation is predicted). We found that for the long-term response, including the interactions between disturbances improved the predictability of the response.

However, for this particular response (return time), two 2-way interactions were found significant and the incorporation of 3- and 4-way interactions did not improve the predictability of the response. Therefore, it appears that the detection of interactions has a large effect on the predictability and further research should either increase the number of replicates for more complex interactions to increase the statistical power or develop a method to take into account this bias.

## Regime shifts, nonlinearity and feedbacks

Nonlinearity is a feature shared by many complex adaptive systems when facing external disturbance (Levin 1998). A consequence of nonlinearity is the historical dependency (i.e., local rules of interaction change as the system develops) that lead to the presence of alternative stable states, and therefore decrease the predictability of the system.

To my knowledge, no empirical studies have manipulated the strength of feedback between the organisms and their environment. In Chapter 4 – the most novel and risky experiment of this thesis – I highlighted that stronger organism-environment feedback affected the nonlinearity and hysteresis in generally the same manner as predicted by theory. This result contradicted – to some extent – the property of complex adaptive systems that nonlinearity causes alternative states due to historical dependency (Levin 1998); but rather than alternate states can be a cause of nonlinearity and hysteresis due to a different configuration of the system (i.e., alternate states) and the delay of the community response and its effect on dissolved oxygen.

To explain the presence of alternate states (extinction or recovery of the predator-prey system) in our experiment, we still lack evidence about the mechanisms. Indeed, we manipulated the gas exchange flux between the atmosphere and the microcosm. However, we did not know the effects of dissolved oxygen on vital rates of the or-

ganisms. To investigate this mechanism, two methods could be considered for further research. First, an experiment using chemostat that would constantly control the gas flow with different temperature regimes could confirm our preliminary results. Second, one of the hypotheses that could explain the recovery of prey and predator was that decreased oxygen concentration would relax the predation rate. Therefore, I would suggest investigating a model where the predation rate could be a function of the oxygen concentration. For example, the distribution of the parameter could become uniform as the oxygen concentration decreases. Then, the probability to have a high or a small predation rate would be randomised.

To another extent, knowing the importance of organism-environment feedback on the community, it could have been interesting to measure over time the nutrients (carbon, nitrogen and phosphorus) in our experiment (Chapter 3) to test whether a positive feedback between the organic enrichment and the community occurred. Indeed, with increased organic matter, overall the abundances increased which involved increased mortality, and the dead material could be used as a new source of organic matter for other organisms.

## General outlook

Populations have three possibilities to cope with global environmental change: migrate, adapt or perish. Therefore, it is essential to study the consequences of global change in natural systems, to determine which populations would be most likely prone to extinction. In freshwater ecosystems migration depends on life history traits. However, for some organisms this is not an option. Therefore, a rapid adaptation to the new environmental conditions is required to prevent extinction. Eco-evolutionary dynamics (i.e., when evolutionary processes can be observed at the same ecological time scale) has to be a complementary approach to identify mechanisms of response to global

change. To conclude, I believe that inter-disciplinary research (e.g., Altshuler *et al.* 2011) is the key to understand global change consequences on natural ecosystems, and to provide sufficient knowledge to managers and policymakers.

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so motivated that seeing me did not discourage you! Frank and Mikael, an amazing post-docs duo that despite some challenges in the post-doc life, you both kept the smile and provided me at least one crucial advice to *keep going*. Dennis, Wilfredo & Rich, my favourite tortoise trio! You brought some tropical sun in the group! And some jealousy to be honest when you were on Aldabra sharing your pictures!! Obviously Maja, I do not know what I would have done with the paperwork if you were not here! And I am sure that you can agree that I am definitely a "P"!! Jacqui & Isabel, the kindest persons who help me with my administrative nightmare! Yves, the *McGyver* of the lab! Thank you for your help and expertise with the protists. And the former group members (Marco, GM, Colette, Clément, Thomas & Aabir). Especially Marco that left a pretty big space (hopefully you left your Ph.D. hat as a good reminder of you!!)

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# CURRICULUM VITAE

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|---|---|
| Name | **GARNIER** |
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## Education

2013–2018 **Ph.D. candidate** - PhD program of Ecology and University Research Priority Programme: Global Change & Biodiversity
Universität Zürich, 8057 Zürich - SWITZERLAND

2011–2013 **M.S. in Evolutionary Biology and Ecology**
Université de Montpellier II, 34000 Montpellier - FRANCE

*2013* "The effects of temperature and nutrient enrichment on body size, population size and oxygen consumption" with Prof. Owen Petchey at the University of Zürich.

*2012* "Do communities depend on their history? Effects of nutrient enrichment and regional species pool on assembly history" with Dr. Isabelle Gounand, Dr. Nicolas Mouquet, Prof. Dominique Gravel and Dr. Sonia Kéfi at the Institute of Science of Evolution, Montpellier (ISEM – *UMR 5554*).

2009–2011 **B.S. in Biology**
Université de Montpellier II, 34000 Montpellier - FRANCE

2007–2009 **Technical diploma in Biological & Biochemical Analyses**
Université du Maine, 53000 Laval - FRANCE

–2007 **General education** (Scientific diploma)
Lycée Sainte Geneviève, 35000 Rennes - FRANCE

## Publications


Altermatt F., Fronhofer E., **Garnier A.**, Giometto A., Hammes F., Klecka J., Legrand D., Mächler E., Massie T.M., Pennekamp F., Plebani M., Pontarp M., Schtickzelle N., Thuillier V. and Petchey O.L., (2015). Big answers from small worlds: a user's guide for protist microcosms as a model system in ecology and evolution. *Methods in Ecology and Evolution*, **6**:218-231.

Pennekamp F., Griffiths J.I., Fronhofer E.A., Seymour M., **Garnier A.**, Altermatt F. and Petchey O.L., (2017). Dynamics species classification of microorganisms across time, abiotic and biotic environments – a sliding window approach. *PLoS One* ; **12**(5):e0176682 10.1371/journal.pone.0176682

**Garnier A.**, Pennekamp F., Lemoine M. and Petchey O.L., (2017). Temporal scale dependent interactions between multiple environmental disturbances in microcosm ecosystems. *Global Change Biology* ; **23**(12):5237-5248.

## Communications

*TALKS:*

Do interactions matter? a fully-factorial experiment of four environmental drivers; **British Ecological Society & Société Française d'Écologie** (Lille (FR), 2014).

Do interactions matter? a fully-factorial manipulation of four environmental drivers on a microbial ecosystem; **Ecological Society of America** (Baltimore (US), 2015).

Predicting effects of multiple environmental changes on community respiration in a microcosm experiment; **Société Française d'Écologie** (Marseille (FR), 2016)

Ecosystem functioning under multiple environmental stressors: stability and predictability; **Behaviour, Ecology, Environment and Evolution Seminar** (Zürich (CH), 2016).

Increasing the number of environmental stressors slows down ecosystem recovery from disturbance; **British Ecological Society** (Liverpool (UK), 2016).

*POSTERS:*

Protists in a changing world: effects of temperature and nutrient enrichment on body size distribution; **British Ecological Society** (London (UK), 2013).

Do interactions matter? a fully-factorial manipulation of four environmental drivers on a microbial ecosystem; **Biology15** (Dübendorf (CH), 2015).

Predicting effects of multiple environmental changes on dissolved oxygen in a microcosm experiment; **British Ecological Society** (Edinburgh (UK), 2015).

Predicting effects of multiple environmental changes on dissolved oxygen in a microcosm experiment; **Biology16** (Lausanne (CH), 2016).

## Grants & Awards

Best presentation at the Conference "Global Change & Biodiversity" in Monte Veritá, CH, 2016. "Simpler is better when predicting effects of multiple environmental stressors in microcosms experiments" (100 CHF)

*Learn from yesterday, live for today, hope for tomorrow.*
*The important thing is not to stop questioning.*
Albert Einstein